THE
AMERICAN NATURALIST

NOTES ON DIFFERENTIAL GROWTH

PROFESSOR JULIAN S. HUXLEY

KING'S COLLEGE, LONDON

IN reviewing the literature of differential growth in animals, I have come across several papers containing data whose analysis has yielded results bearing on the subject. As the authors of the papers have not considered the bearings of their facts on differential growth, I am summarizing them here.

PART 1. GROWTH-GRADIENT IN THE ABDOMEN OF CRABS

A paper by D. Atkins (1926), gives scale drawings of different stages of the female pea-crab (*Pinnotheres pisum*). I have taken measurements of her drawings and reduced them to absolute figures in accordance with the magnifications she gives. The result is given in Table I.

Owing to the convexity downwards of the abdomen, which increases rapidly in later stages, it is probable that the measurements of segments are sometimes too small. This will apply very slightly to the length of the segments towards the tip, considerably to those near the base (nos. 4 and 3). It will apply also to the breadth-measurements in later stages, especially of the broader segments. This is, however, not likely to make more than 10 per cent. difference to the measurements.

To give us an idea of the differential growth of different parts, we can take the ratios of the measurements of the last stage with complete measurements (stage 5; ♀ 7) to those of the smallest stage (stage 1; ♀ 1); this will give

TABLE I

Pinnotheres pisum. ALL MEASUREMENTS IN MM.

	♂	♀1	♀2	♀3	♀4	♀5	♀6	♀7	♀8
Carapace width	5.98	2.10	4.90	4.44	5.98	6.22	8.72	10.14	{ about
Carapace length	6.06	[2.25]*	5.06	4.44	5.92	6.14	8.30	9.30	} 14.
Abdomen length	3.58	1.406	3.02	2.94	3.96	4.54	6.92	9.10	....
Length of separate segments of abdomen 3	.706	.369	.48	.38	....	.46	....	....	....
.. 4	.623	.264	.52	.54	.74	.92	1.24	1.66	....
.. 5	.670	.223	.76	.48	1.00	1.00	1.90	2.22	1.80
.. 6	.670	.230	.60	.66	1.06	.82	1.92	2.28	2.87
Telson 7	.777	.176	.48	.76	.86	.92	1.34	2.04	2.77
Breadth of separate segments of abdomen 2	....	.845	....	....	....	....	....	9.4	....
.. 3	1.920	.697	1.70	2.52	5.00	4.08	7.60	9.4	....
.. 4	1.575	.553	1.54	2.74	5.62	4.24	8.24	10.92	15.35
.. 5	1.270	.459	1.26	2.76	5.72	c4.10	8.48	10.80	15.46
.. 6	1.035	.405	1.06	2.50	5.21	c3.75	7.88	10.00	14.63
Telson 7	.800	.257	.70	1.84	3.56	c2.50†	5.30	7.30	11.19
	Stage 1	Stage 1	Stage 2	Stage 3a	Stage 3b	Stage 4	Stage 5	Stage	

* The length can not be measured, as the upper surface is not figured. It may be taken as 2.25.

† The last three measurements are only approximate, owing to the overlap of the limbs in the drawings.

a measure of the increases of the different parts. We can further compare the rate of other parts relative to that of

TABLE II

RELATIVE GROWTH OF PARTS OF ABDOMEN IN *Pinnotheres*

	car.br.	car.l.	abdomen segments l.				abdomen segments br.				
			4	5	6	7	3	4	5	6	7
Increases of parts (ratio of measurements) stage 5: stage 1	4.83	4.13	6.29	9.96	9.92	11.59	13.49	19.75	23.55	24.7	28.4
Ratio of increases to increase of carapace width	1.00	0.86	1.30	2.06	2.05	2.40	2.79	4.09	4.88	5.12	5.88
Growth coefficients (relative to carapace length)	1.11	....	1.30	1.62	1.62	1.73	1.83	2.10	2.23	2.26	2.36

carapace-breadth by taking the increase of the latter during this period as unity and reducing the other increases (ratio, stage 5; stage 1) proportionately.

For comparison with other forms, however, we can obtain an approximate value of the growth-coefficient (k) from the formula

$$k = \frac{\log y_1 - \log y_0}{\log x_1 - \log x_0}.$$

x is here taken as carapace *length,* x_1 at stage 7, x_0 at stage 1. The figures for k are given in the last line of the table.

Before considering the abdomen, we may note that the carapace begins longer than broad, but owing to the higher breadth-growth its proportions are finally reversed. In the abdomen, the first thing to notice is the extremely high relative growth in width of certain segments, up to nearly six times as fast as the increase of the carapace. These are among the highest figures yet found for differential growth.

More interesting, however, is the demonstration of a growth-center and growth-gradient. In previous papers, (Huxley 1927, etc.) I have shown that where an organ is growing more rapidly than the body (heterogonic growth) it appears never to be growing uniformly throughout its length, but to have a growth-center where growth is most intense, with the intensities of growth grading down steadily in neighboring parts, forming a growth-gradient, single if the growth-center is terminal in the organ, double if it is not terminal. In markedly heterogonic organs, the growth-center appears to be usually terminal or sub-terminal, in slightly heterogonic organs, central or nearer the base.

In this case, the growth-center is clearly terminal (if we consider the segments as units, which is all we can do). This is true both for length and for breadth; but the gradient is much steeper for breadth.

This is hardly what inspection of the drawings would suggest, since the central parts of the abdomen (seg-

ments 4 and 5) though not broadest in the youngest females, become the broadest in the old females, being much broader than segment 7. They achieve this because, although their rate of increase is less, their initial breadth was correspondingly greater.

When we take the ratio of the relative growth in breadth to that in length, as measured by the ratio of the growth-coefficients, for the separate segments of the abdomen, we find the following result. Segment No. 4, 1.62; No. 5, 1.46; No. 6, 1.40; No. 7, 1.36. There is thus a steady decrease, indicating that between breadth-growth and length-growth there exists an orderly relation. Although the last segment is growing the most rapidly in breadth, and although its breadth-growth is much higher than its length-growth, it is deforming itself in the transverse dimension less rapidly than are the more proximal segments. Thus here we have the growth-gradient for length steeper than that for breadth, although at a lower absolute level.

A paper by Sasaki (1928) makes similar measurements for a Japanese shore crab (*Telmessus cheiragonus*), both for males and females. He finds that the abdominal segments grow according to the simple heterogony formula $g = bx^k$, where y in the organ studied, x a standard part (here the carapace length), and b and k are constants. The value of k of course gives the ratio of the growth of the part to that of the standard. We may conveniently tabulate all the values of k for the breadth of the several abdominal segment of both sexes and also for total abdominal length; (the range covered is carapace length 11 to 77 mm).

TABLE III

TELMESSUS CHEIROGONUS: GROWTH RATIOS IN THE ABDOMEN

Breadth of segments	2	3	4	5	6	7	Total length
♂	1.10	1.10	*1.14*	*1.14*	1.10	1.00	1.06
♀	1.19	1.23	1.32	1.34	*1.36*	1.15	1.11

The lengths of the separate segments were unfortunately not taken. In *Telmessus* the carapace width increases less rapidly than the carapace length ($k = 0.95$). If the increase between smallest and largest specimens be taken, and the ratio of the increase of the most rapidly broadening abdominal segment to that of the carapace length be calculated, we find it is very small in comparison with the value for *Pinnotheres:* 1.9 for the 6th segment of *Telmessus* as against 5.88 for the 7th segment of *Pinnotheres*. (In the male, the highest similar ratio is 1.45 for the 5th segment.)

Thus in both sexes of *Telmessus* there exist growth-gradients in the abdomen. That of the male is not at all steep, and has its center in the 4th and 5th segments; that of the female is twice as steep, and has its center in the penultimate segment.

It is interesting to find the steeper female growth-gradient with its center more terminal than that of the male, but less terminal than the extremely steep female gradient in *Pinnotheres*.

There remains one point to be discussed. Atkins describes two forms of females in stage 3, one with relatively long and narrow abdomen (represented by ♀ No. 5), the other with shorter and broader abdomen (represented by ♀ No. 4). So far as one can gather from the few specimens available, the development of the female abdomen is somewhat as follows. Several moults may take place during stage 1, the carapace-width of which ranges from 2.1 to about 5.0 mm (Atkins gives 4.9 mm as the maximum, but her Fig. 3, according to the magnification given, is 5.06 mm in carapace-width). During this phase, there is relatively little differential growth within the abdomen. Atkins states that there is *no* change of form, but a comparison of her small and large crab of this stage (♀ 1 and ♀ 2) indicates that this is not strictly accurate: the differential growth of the segments has already begun, the ratio of their breadth-increase to that of the carapace ranging from 1.05 (3rd segment) to

1.13–1.19 (other segments). The measurement-errors are here so relatively large that it is unwise to try to establish any gradient in the segments.

The onset of rapid differential growth may begin at very various absolute sizes. If it takes place when the animal is of about $3\frac{1}{2}$ mm carapace width we should (following Przibram's rule of the approximate doubling of volume of Crustacea at each moult) at the next moult get a crab of about the size of our ♀ 3. If, on the other hand, the onset of this phase is delayed until 4.5 to 5 mm carapace-width, we shall get an animal of the size of our ♀ 5. Accordingly, I would regard stage 3b of Atkins as being a very late stage 2. In actual fact the proportions of the abdomen and its segments are quite close to those of stage 2 (maximum breadth of abdomen 68.1 per cent. of carapace-breadth in stage 3b, as against 62.3 per cent. in stage 2, 34.7 per cent. in stage 1 (♀ 2) and 95.7 per cent. in stage 3a). During the period between stage 1 and stage 3 (Atkins stage 3a), the relative growth of abdomen-breadth must be very high (k in the heterogony formula over 2); after this, judging by the three points available, a definite but moderate heterogony with k about 1.3 appears to supervene. Similar variations in the time of onset of heterogony have been found with the male chela of Maia (Huxley, 1927), the female abdomen and the male chela of *Inachus* (Shaw), and is doubtless not uncommon. Atkins herself states (p. 477) that one stage 1 female was found with more approximation to the female form of abdomen than usual: and also that crabs have been found "with the abdomen reaching as far forward as in stage 4, but as wide as in stage 5, and *vice versa.*" Clearly a marked heterogony in breadth and a minor heterogony in length both occur; these are (a) continuous processes, broken up into steps by the accidents of moulting; (b) the time of the onset of one is probably the same as that for the other, but this may vary relative to the size of the animal; and (c) the quantitative relation between length-heterogony and breadth-heterogony

may vary from individual to individual (just as it does in man, giving asthenic and pyknic types.)

The only male figured is not far from the smaller female of stage 1 in its abdominal proportions, but all save one of its segments are inferior in proportionate breadth (per cent. of carapace); and while its basal segments are inferior in proportionate length, its distal segments (5–7) are superior.

It is clear that a detailed study of the relative abdomen growth of this species, using really accurate measurements and reasonable numbers, would be of interest.

Part 2. Negative Growth-centers in the Walking Legs of the Racing Crab *Ocypoda ceratophthalma*

In a recent paper, Cott (1929) describes the biology of this typical semi-land crab. In it he gives a few measurements, showing that it has relatively very long legs in relation to its running habits, and that its young are precocial and, accordingly, like those of the ungulates, have relatively longer legs than the adults.

Measurements made by Hammond (See Part 3 of this paper) on the growth of the limb-segments in sheep show that during the marked relative decrease (negative heterogony) of these organs during the early years of life, there is a *reversed* growth-gradient, with center of least growth in the carpus and tarsus, while the proximal segments (humerus and femur) are growing more rapidly than any other parts of the limb. Thus, when as in crustacean chelae there is marked positive heterogony, the center of maximum growth is sub-terminal (propus); and when as in these Sheep limbs there is negative heterogony, the center of minimum growth is also sub-terminal. This suggested that the growth-gradients of positively and negatively heterogonic organs would be similar in form, but with sign reversed throughout, and prompted me to search for further examples. *Ocypoda* was such an example, and I wrote to Mr. Cott asking if he had measurements of the separate segments of the

walking limbs, in addition to the total lengths which alone
are given in the paper. He kindly took measurements on
7 specimens, which were all he had available, and put
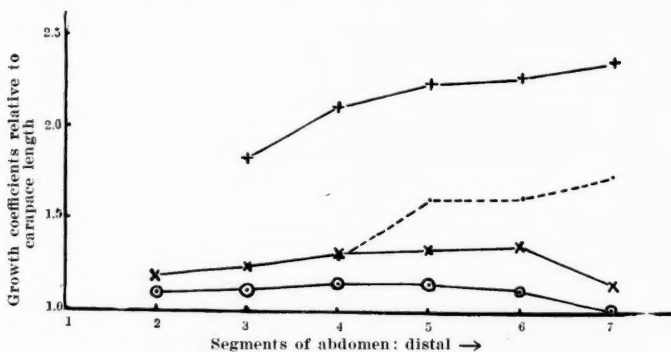them at my disposal.

Fig. 1.   Growth-gradients for abdominal breadth in ♂ *Telmessus*, ⊙;
♀ *Telmessus*, ✕; ♀ *Pinnotheres*, +; and for abdominal
length, ♀ *Pinnotheres*, dotted line.

Now in *Eupagurus prideauxi*, the only crustacean on
which I could find detailed measurements of the separate
segments of the pereiopods, (Bush 1930), these limbs
show distinct but slight positive heterogony; and in them
there is a moderate double gradient with growth-center in
the merus.

After plotting Cott's figures in various ways, I came to
the conclusion that the best way to treat them for my
purpose was to take the mean for the two smallest crabs,
of 3.7 and 4.0 mm carapace length, both immature, and
for the two largest crabs, of 28.5 and 30 mm carapace
length, one ♀ and the other ♂ (sex appears to make little
difference to the relative size of the pereiopods in this
species), and to see what the increase of the various seg-
ments was for this increase of carapace length. The re-
sults are tabulated below. The growth coefficients (k)
relative to carapace length have been calculated as in Pt.
1 for two of the limbs.

TABLE IV

Ocypoda WALKING LEGS

(Measurements in mm)

	Small	Large	Ratio Large : small	Growth coefficients (k)
Mean carapace length	3.85	29.25	7.60	
Pereiopod 1, total 1	13.45	87.50	6.48	0.93
Segments I–III	1.85	14.75	7.97	1.03
" IV	4.35	25.75	*5.92*	0.88
" V	1.60	10.75	6.73	0.94
" VI	2.80	19.25	6.88	0.95
" VII	2.85	17.25	*6.06*	0.89
Pereiopod 2, total 1	15.15	96.25	6.35	
Segments I–III	1.95	16.25	8.34	
" IV	5.00	29.50	*5.90*	
" V	1.70	11.00	6.48	
" VI	3.45	21.75	6.31	
" VII	3.05	17.75	*5.83*	
Pereiopod 3, total 1	14.45	93.00	6.50	
Segments I–III	1.80	15.00	*8.34*	
" IV	4.80	28.75	*5.99*	
" V	1.70	11.50	6.77	
" VI	3.40	21.25	6.25	
" VII	2.75	16.50	*6.00*	
Pereiopod 4, total 1	11.15	70.00	6.28	0.91
Segments I–III	1.60	11.75	7.35	0.98
" IV	3.85	21.50	*5.58*	0.85
" V	1.35	9.75	7.22	0.97
" VI	2.25	15.00	6.67	0.93
" VII	2.10	12.00	*5.72*	0.86

For the cheliped different measures must be taken, as it had been lost in the large ♂, and as this organ may be expected to be less well-developed in the ♀. I therefore take its increase from the same two small specimens to the largest complete ♂, with carapace-length 16 mm. We then have the result shown in Table V.

These results are thus given graphically in Fig. 2. Too much reliance can not be placed upon the precise values of k, but the fundamental similarity of the gradients for the walking legs is clearly shown and their fundamental difference from that for the chela. The cheliped behaves entirely differently from the other limbs. It is practically isogonic with the carapace; and has its growth-center in the merus, like the pereiopods of

TABLE V

Ocypoda ♂ CHELA

	Small	Medium	Ratio Medium : small	Growth coefficients (k)
Mean carapace length	3.85	16.0	4.16	
Cheliped, total 1	9.35	39.0	4.17	1.00
Segments I–III.................	1.70	7.0	4.11	0.99
" IV	2.20	10.0	*4.55*	1.06
" V	1.40	6.0	4.28	1.02
" VI (to tip of 'finger')	4.05	16.0	3.96	} 0.96
" VII	not separately measured			
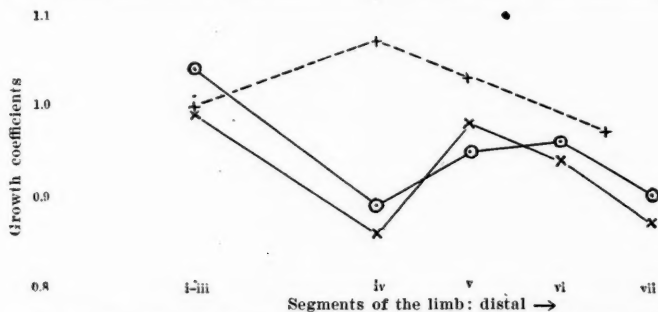
FIG. 2.   Growth-gradients of the 1st (⊙) and 4th (✕) pereiopods and the cheliped (dotted line) of *Ocypoda ceratopththalma*. (The last point for the cheliped is placed between segments VI and VII, since the measurement was made to the tip of the propus finger, which includes the length of VII.).

Eupagurus, and like the cheliped of that animal so long as it is but slightly positively heterogonic.

The pereiopods, on the other hand, show definite though not large negative heterogony: and their "negative growth-center" (center of minimum growth) is invariably in the same segment which in the slightly positively heterogonic pereiopods of *Eupagurus* is the positive growth-center, the merus.   The idea with which we started is thus in this respect confirmed.

There is not, however, a regular reverse growth-gradient from this point up in both directions; the ratios, after rising from the carpus, sink again for the propus and

dactylus (except in ppd 1, where they rise slightly for the propus).

This may have something to do with the fact commented on by Cott (p. 763) that in *Ocypoda* not only are the walking legs relatively long, in comparison with less active forms, throughout life, in spite of their negative heterogony relative to the body, but that this length has been achieved largely by a lengthening of the propus and dactylus.

The total length of the second walking leg in a specimen of the slow-moving crab *Sesarma meinerti* of carapace breadth 41 mm was 748 mm, as against 100 mm for that of an *Ocypoda* of the same carapace length. The ratio of the *Ocypoda* to the *Sesarma* leg is 1.34; but the ratio of the lengths of the various segments is as follows (Cott's Table I) : I–III, 1.22; IV, 1.33; V, 1.05; VI, *1.57;* VII, 1.45. It would appear that to achieve the excessive leg-length, a shift in the distribution of growth-intensity, presumably in very early life, has taken place, giving a second growth-center in the propus during the period of very rapid growth. The same phenomenon is witnessed in the right male cheliped of *Eupagurus,* when it changes from slight to marked positive heterogony, (Bush, *l.c.*).

In addition, the dactylus is extremely slender and tapering; thus a purely linear measurement gives too high a value for its total growth-intensity in a way which does not apply to the more or less quadrangular oblongs of propus and merus; a weight-measurement is really required.

The only other point which needs noting is the curious fact that in pereiopods 1–3 the relative length-growth of segments I to III is actually higher than that of the body, although the limb as a whole shows negative heterogony. This phenomenon again should be investigated on the basis of weight-measurement.

It is clear from these preliminary results that the detailed investigation of two not too-distantly related

forms, one showing positive, the other negative heterogony of a corresponding part, would be of considerable value.

Part 3. Negative Growth-gradients in the Limbs of Sheep

Hammond (1927 and 1929) has published certain interesting facts concerning the growth of the separate parts of the skeleton in sheep, indicating definitely the existence of growth-gradients in the limbs; but he has only given the relative weights (relative to the cannon-bone) not the absolute weights of the different parts.

He has kindly put his original data for the Suffolk breed of sheep at my disposal, and the following pages represent an analysis of these.

Measurements exist for 15 specimens, from new-born to adult, some males, some females, some castrated males. I have concerned myself only with the data for the different parts of the limb-skeleton (including limb-

TABLE VI

Weights (grams) of Selected Parts of the Skeleton in Specimens of Suffolk Sheep, from Hammond's Data. The Roman Figures Denote the Reference Numbers of Hammond's Specimens; Where More Than One Such Number Appears, Means Have Been Taken. k_1, k_2 and k_3 Denote the Values of the Growth-Coefficients of the Several Parts, Relative to the Vertebral Column, for the Three Selected Phases of Size-Increase

	II	IV	k_1	V + IX + X	k_3	XV + XX	k_2
vertebrae	100.1	256.4		690.0		2075.5	
scapula	6.2	19.2	1.20	66.2	1.25	219.0	1.09
humerus	12.3	36.6	1.16	99.2	1.01	244.5	0.82
radius + ulna	12.08	35.7	1.15	78.7	0.80	195.8	0.84
carpals	3.35	9.4	1.10	18.2	0.67	32.8	0.53
metacarpals	8.15	24.5	1.17	44.5	0.60	85.0	0.59
pelvis	17.6	33.0	0.67	185.0	1.74	512.0	0.94
femur	16.0	49.6	1.20	126.5	0.95	236.8	0.57
tibio-fibula	15.18	45.8	1.17	105.3	0.84	209.3	0.62
tarsals	8.88	20.8	0.90	41.7	0.70	63.5	0.38
metatarsals	7.65	23.3	1.18	43.2	0.62	74.3	0.49

girdles) and as standard to plot these against I have taken his figures for the weight of the whole vertebral column: See Table VI.

Fig. 3 represents a double-logarithmic plot of the different parts of the fore-limb (omitting the figures for the
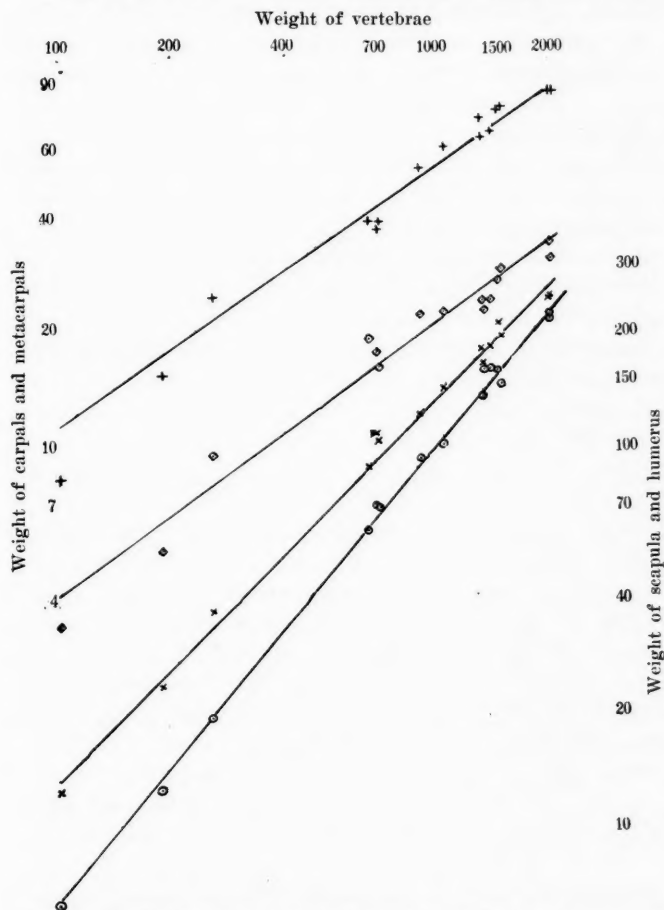

FIG. 3. Double logarithmic plot of weights of scapula ⊙, humerus ✕, carpals ◊, and metacarpals +, against weight of vertebrae in 15 Suffolk sheep (Hammond's data). The lines for each set of points are only inserted as visual aids.

radius-plus-ulna, as these overlapped those for the
humerus and the scapula in a confusing way). It will be
seen that there is, as one would expect, considerable
variation, but that the points tend to be distributed along
straight lines, indicating the existence of constant dif-
ferential growth-ratios for the parts concerned. The
first three points, however, especially as regards the
carpals and metacarpals, are rather aberrant. This may
perhaps be accounted for by the fact that these three
animals, though of very different absolute size, were all
of the same age (new-born); the smallest was one of trip-
lets, the largest was a single birth, the intermediate
specimen one of twins.

The corresponding figures for the parts of the hind-
limb skeleton are similar, but a little more irregular,
notably for the three first animals.

In order to obtain a graphic presentation of the growth
gradients in the limbs, I have calculated the value of k,
the growth-coefficient of the part, in the heterogony
formula $y = bx^k$, from the formula

$$k = \frac{\log y' - \log y}{\log x' - \log x},$$ where y' and x' are the values for the

part at a higher absolute size, y and x those at a lower
value. I have calculated the coefficients for three por-
tions of the size range: (a) from the smallest to the
largest of the three new-born specimens; (b) from the
largest of the three new-born specimens to the mean
value for the three specimens with vertebral column
weight close to 700; (c) from this latter point to the
mean for the two largest specimens.

The relevant figures are given in Table VI.

Graphic representation of the results for all three
phases in the fore-limb, and of those for the two last
periods in the hind limb, are given in Fig. 4.

If we first look at the graph for the central period,
we find in both extremities a remarkably smooth growth-
gradient, with center of minimum growth in the sub-
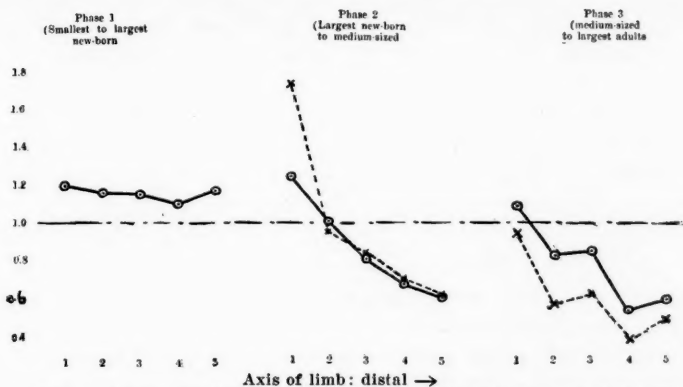terminal region (unfortunately Hammond has not de-

FIG. 4. Growth-gradients of sheep limbs in 3 size-phases. The distal end of the limb is to the right. Forelimb, solid line; hindlimb, dotted line. In each phase, 1 = limb-girdle; 2 = humerus; 3 = tibio-fibula; 4 = tarsals or carpals; 5 = metatarsals or metacarpals.

termined the weights of the phalanges) and a steady increase of growth-ratio as we pass towards the body and in to the girdle.

The same type of gradient is seen in the last phase, but is somewhat irregular for both limbs. Such irregularities, however, are only to be expected with so few specimens. In general, the results confirm the views previously set forth (Huxley 1927), *viz.,* that when an organ is growing at a markedly different rate from the body as a whole, it will be found to show a growth-gradient. When it is growing faster than the body, we shall expect to find the growth-center or point of maximum growth at or near the distal end of the limb: this is realized in the chelae of Crustacea, and the abdomens of female Brachyura. Here, however, the limb exhibits negative heterogony: and we find a reversal of the sign of the growth-gradient, with center of minimum growth near the distal end.

It will be seen that the pelvis is growing faster than the vertebral column as a whole in both cases. This, Hammond's data further show, is associated with a high growth-rate of the lumbo-sacral region of the backbone as against the other regions.

The relative growth-rate of the whole limb sinks between the second and third phase; in both cases the limb as a whole is growing slower than the backbone. In the first phase, the growth-gradient is quite different, approximating to a straight line. Furthermore, the growth-coefficient of the limb as a whole, and of all its parts, is higher than that of the vertebral column. We may suppose that the small size of the smallest individual, due to its being one of triplets, has prevented it completing the previous growth-phase which is usually finished during intra-uterine life. During this, the limbs must clearly be exhibiting positive heterogony, thus causing the young animal to be born with disproportionately long limbs. Further, the growth of the scapula in this phase is lower than in the next phase. The growth-gradient for the hind limb in this phase (not reproduced in the figure) is more irregular, but in general similar; the value for the growth-ratio of the pelvis is very low.

We may therefore suppose that the sequence of events is somewhat as follows:

(1) Strong positive heterogony of the limbs, which then presumably possess a positive growth-gradient with growth-center near their distal ends.

(2) Increase of pelvis growth, and decrease of terminal limb growth, resulting in a flattening of the gradient (stage represented in our "first phase."

(3) Continued increase of pelvic (and presumably lumbo-sacral) growth, onset of marked negative heterogony of the limb. Result, a steep growth-gradient with negative growth center distally (our "second phase").

(4) Continued negative heterogony of the limb, reduction of pelvic growth (our "third phase").

Mr. Hammond points out that different bones grow in different ways. Most bones grow by a combination of epiphyseal growth and general thickening, but the carpals and tarsals have no epiphyses and must rely solely on the second method. Then in the radius and ulna the epiphyseal growth continues longer than in the humerus. It is possible that minor discrepancies in the growth-gradients could be explained by reference to such facts.

In conclusion, I would like to thank Mr. Hammond for kindly allowing me to utilize his data, which at the moment of writing are unpublished, although they are shortly to appear in a book.

Part 4. Distribution of Growth-activity in the Metamorphosing Herring

In a recent paper Ford (1930) has discussed the changes in proportion in the herring during its transformation from the eel-shaped larva of rather less than 35 mm to the adolescent fishlet, with more or less the adult form, of 55 mm or more in total length. He finds that the head increases rapidly in relative size throughout the process, as does the ventral region between anus and end of trunk (root of tail) and the dorsal region from the first ray of the dorsal fin (D_1) and the end of the trunk.

The ventral distance from the pelvic fin to the anus, on the other hand, and the dorsal distance from hind end of head to D 1 decrease markedly in relative size; the ventral distance from hind end of head to pelvics decreases slightly in relative size. The increases and decreases are taken relative to body-length without tail. The tail stays of approximately the same proportion throughout, one seventh of total length. As a result of these complex changes, D 1, the first dorsal fin-ray, moves forward relative to the vertebral column, the pelvic fins backward; whereas in the larva the pelvics are 6 vertebrae anterior to D 1, in the metamorphosed fishlet they are 3 vertebrae posterior to it.

It occurred to me that it would be of interest to analyse the data from the point of view of relative growth-ratios, but the data presented in the paper did not permit of this analysis. However, Mr. Ford kindly put his original data at my disposal and discussed several difficulties with me.

It speedily became evident that only animals from samples of approximately the same date were comparable (see below). The following remarks accordingly

apply to 183 animals caught in the Tamar on 2/5/29 and
18/5/29 respectively. Relative head-length appears to
be much the best criterion of metamorphic stage, and the
animals were accordingly grouped into 12 classes from
10 per cent. to 21 per cent. head length, the means for the
various regions of the body determined for each class,
and plotted on double logarithmic paper against body-
length. The results are shown in table VII and Fig. 5. On
the whole, close approximations to straight lines are ob-
tained, showing that the regions of the body are main-
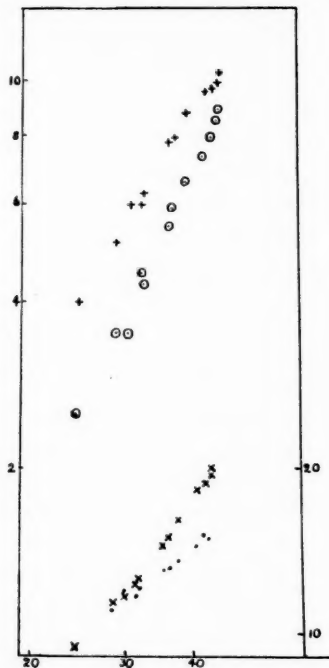taining constant growth-ratios during metamorphosis:

FIG. 5. Double logarithmic plot of the lengths of various regions of 182
metamorphosing herring grouped in 12 classes by relative head-length.
Abscissa, total length without tail (A–E in Fig. 6a) ⊙ head length. (A–B).
+ anus to root of tail (H–E). ✕ 1st dorsal fin-ray to root of tail (C–E).
⊙ pelvics to anus (G–H). The scale on the left refers to the first two
measurements, that on the right to the last two. Measurements in mm.

deviations, if present, are mainly in the extreme end-classes.

<div align="center">TABLE VII</div>

<div align="center">Measurements (mm.) of Different Regions of the Body in 182 Meta-morphosing Herrings Arranged in Classes by Relative Head-Length</div>

Class	Head 1. % of A–E	M	Length to root of tail (A–E)	Head 1. (A–B)	Pelvies to anus (G–H)	Anus to root of tail (H–E)	1st dorsal to root tail (C–E)	Head to pelvies (B–G)	Head to 1st dorsal (B–C)
I	10.0–10.0	2	24.0	2.5	8.0	4.0	9.5	9.5	12.0
II	11.0–11.9	7	29.9	3.5	8.5	6.0	11.7	11.9	14.7
III	12.0–12.9	12	28.2	3.5	8.6	5.1	11.3	11.0	13.4
IV	13.0–13.9	16	31.6	4.3	8.9	6.3	12.7	12.1	14.6
V	14.0–14.9	19	31.1	4.5	8.9	6.0	12.3	11.7	14.3
VI	15.0–15.9	10	35.0	5.5	8.6	7.8	14.4	13.1	15.1
VII	16.0–16.9	18	35.6	5.9	8.6	7.9	15.0	13.2	14.7
VIII	17.0–17.9	18	37.3	6.6	8.3	8.8	16.1	13.6	14.6
IX	18.0–18.9	29	40.1	7.4	8.4	9.7	18.3	14.6	14.4
X	19.0–19.9	29	41.6	8.0	8.6	9.8	18.8	15.2	14.4
XI	20.0–20.9	18	42.4	8.6	8.7	10.1	19.5	15.0	14.3
XII	21.0–21.9	4	42.8	9.0	8.3	10.5	20.0	15.0	13.8

The letters refer to Fig. 4. A–B is head length; G–H, pelvies to anus; H–E, anus to root of tail; C–E, first dorsal to root of tail; B–G, end of head to pelvies; B–C, end of head to first dorsal.

As results we can say that the measured regions of the body fall into three groups:

(A) Those with positive heterogony relative to body-length.
  (1) Head-length (A–B); growth ratio about 2.25.
  (2) Ventral distance from anus to end of trunk (H–E); growth-ratio about 1.65.
  (3) Dorsal distance from 1st dorsal fin ray (D1) to end of trunk (C–E); growth-ratio about 1.42.
(B) Those which show absolute increase of size but negative heterogony.
  (4) The ventral distance from hind margin of head to pelvic fins (B–G); growth-ratio about 0.77.
(C) Those which show no absolute increase of size, but remain stationary or even regress.
  (5) The dorsal distance from hind end of head to D1 (B–C).
  (6) The ventral distance between pelvic end fins and anus (G–H).
Both of these, omitting the first size class, show *negative growth-ratios*, of about—0.05 in No. 5 and rather more in No. 6.

These results are shown graphically in Fig. 6a. In addition, by examining Ford's drawings it would ap-

pear (a) that the ventral surface of the head grows more than the dorsal, resulting in the shift of the mouth from ventral to dorsal of the anterior end; (b) that the region in front of the eye grows most rapidly of any in the head, and the eye itself next, while the region from eye to hind margin of head is growing only about as fast as the body as a whole; (c) that the relative length of the dorsal fin remains approximately constant, which means that it is the region posterior to this which is responsible for the positive heterogony of the dorsal distance D 1—end of trunk; (d) that in the region from anus to end of trunk, the major amount of heterogony is due to the growth of the anal fin region, whereas the region behind this remains almost constant relative to trunk length. In these points, however, Mr. Ford tells me that his drawings do not pretend to great accuracy, so that quantitative results can not be expected.

On the basis of this analysis, I have constructed Fig. 6, which gives a rough idea of how growth-intensity is distributed to various regions of the dorsal and ventral surface of the metamorphosing herring. We may call this the animal's growth-profile. The ordinates denote growth-ratios—the ratio of the length-growth of the part in question to that of the length-growth of the body as a whole, as denoted by the exponent k in the heterogony formula [part $= b.$ (body)^k]. Values over 1 indicate positive heterogony, below 1 negative heterogony. O indicates that the part is stationary in absolute length (I have not attempted to indicate absolute decrease in length).

It will be seen that the ventral surface shows higher growth-ratios than the upper. The shift of pelvic and dorsal fin relative to each other is caused by the presence of a ventral zone of growth anterior to the pelvics, of no-growth posterior to them, whereas the opposite is the case dorsally with regard to D 1. The anterior end of the head seems to be much the most rapidly growing region of the whole body, and more so on the ventral than on the dorsal side.
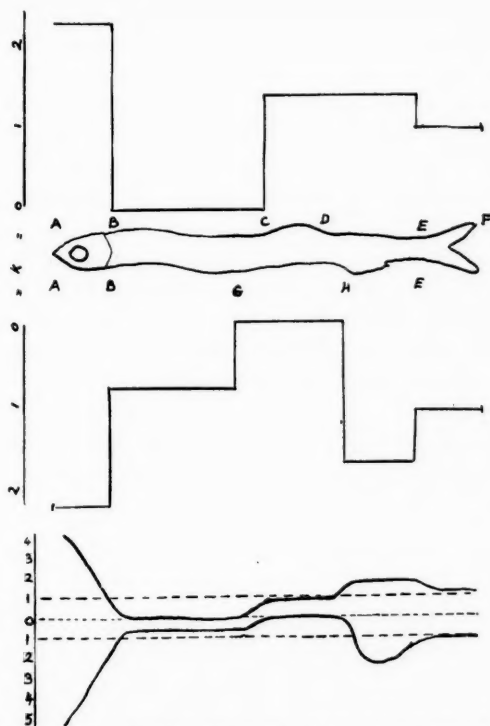
FIG. 6 (a). Outline drawing of young herring at onset of metamorphosis, from Ford (1930). Superimposed on it is its growth profile, constructed from the growth-coefficients for the various regions of the body, dorsal and ventral, as determined from the measurements in Table 6. (b) Growth-profile (on a smaller scale) of the same animal, constructed by taking into account drawings of different stages as well as measurements (see text).

The figure is of course only a first approximation to the truth. By making a detailed investigation of the length-changes of other regions, especially of the separate vertebrae, very interesting results could be obtained, and we might expect to obtain a series of growth-gradients instead of these regions of abruptly differing growth ratios.

It remains to be noted that in a sample of metamorphosing herrings taken on July 16, 1928, the proportions of the different regions were rather different. There

were some smallish animals with head-length taking up
23 and 24 per cent. of body-length. In these, head-length
was right above the line determined for the 1929 samples.
There were also a considerable number of very large ani-
mals, up to 90 mm body-length, with head-length only 19
or 20 per cent. In these, the head-length was well below
the continuation of the 1929 line, and other proportions
indicated that they were only about half as far metamor-
phosed as 1929 animals of about half their body-length.
Presumably temperature and food can markedly alter
the size and proportions at which metamorphosis is
initiated, as is the case of course in, *e.g.*, Amphibian
larvae. Here again an interesting field is open for
further studies.

I must conclude this section with my best thanks to Mr.
Ford for his kindness in putting his data and his ex-
perience at my disposal.

PART 5. THE NORMAL AND REGENERATING CHELA OF *Portunus sayi*

Zeleny (1908) gives interesting data on the normal
growth and the regeneration of the chela of the Gulfweed
crab, *Portunus sayi*. He gives the length of the propo-
dite of the right chela and also the length of the carapace
for a number of specimens from about 3 to about 15 mm
carapace length. These results, extracted from his
tables and tabulated by me according to body-length
classes, are given in Tables VII and VIII, and are
plotted on a double logarithmic grid in Fig. 7. It is seen
that they give an excellent approximation to a straight
line, from the slope of which we can calculate the growth-
coefficient (k) of the chelar propus length to be about
1.15. There is further an indication of a double sinuosity
in the curve, the points up to carapace-length about 5.5
showing a slightly higher growth-ratio, those from 5.5 to
9.5 a slightly lower one. This may perhaps be associated
with breeding and non-breeding seasons, which we know
to affect the growth of the male chela of Inachus (G. W.
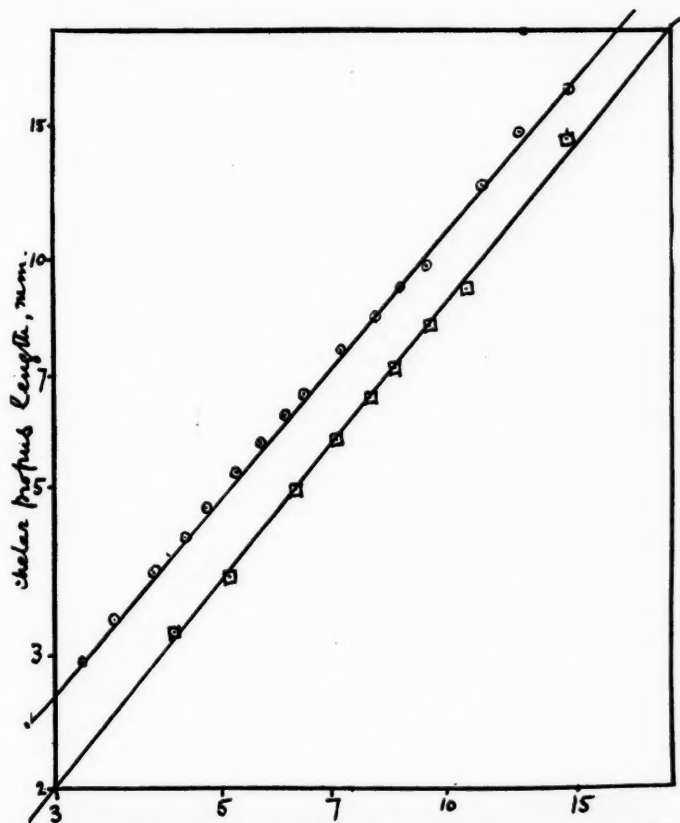Smith 1905). In passing, the last column of Table VII

Fig. 7. Double logarithmic plot of normal (⊙) and regenerating (□) right chelar propus of *Portunus sayi* against carapace-length (data of Zeleny 1908).

shows that there is no significant change in the ratio between R/L than with growth.

Zeleny also undertook a large number of experiments on regeneration. These are very valuable for our purpose, as all the results are comparable in every respect except size of animal at operation. After first ascertaining that the chief essential factor regulating amount of regeneration was moult-period, irrespective of the absolute amount of time occupied, he proceeded to do all his operations immediately after one moult, and all his mea-

surements of amount of regeneration immediately after the next.

His data enable us to plot the amount of chela (propus length) regenerated during one instar against body-size (carapace-length). The points thus determined also fall onto a straight line on the double logarithmic grid. As regeneration is never complete after a single moult, this line is of course below that for the normal claws. In addition, however, it shows a slightly but distinctly different slope, with $k =$ about 1.20. Thus the amount of chela regenerated after total amputation during a single instar stands in a definite fractional relation to the normal size of the claw for the body-size at the moult ending that instar; but the fraction increases slightly with absolute size of the animal. In other words, large animals regenerate a slightly greater proportion of their chelae than do small ones. Assuming the slope of the lines in the figure to be correct, the smallest regenerating crabs regenerate less than 78 per cent., the largest ones over 82 per cent. of the normal chela for that body-size. The difference is not large, and experiments should be conducted on a species with a larger absolute size-range to see whether it is really significant. If established, it would appear to be surprising, but might be accounted for according to the well-known principle that the rate of regeneration increases with the amount removed.

Leaving on one side the question of this possible small alteration in the regenerated fraction with change of absolute size, we see the strict quantitative relationship between the process of normal and regenerative growth, which Przibram has repeatedly stressed.

Zeleny himself stresses the facts (a) that relative chela-size increases with absolute body-size and (b) that "the proportion between the amounts of regeneration of a chela and the length of the chela in uninjured individuals of the same size is a constant uninfluenced by the size of the animal," but I have felt this note to be useful in pointing out that both normal and regenerating chelae conform in their growth to the laws of constant coeffi-

TABLE VII

SIZE OF PROPUS OF CHELAE IN UNOPERATED *Portunus sayi*, ARRANGED BY
CARAPACE-LENGTH CLASSES. FROM TABLES OF ZELENY (1908)

Class	No. of specs.	Mean length (mm) of			Ratio R/L chelar propus, per cent.
		carapace	L. chelar propus	R. chelar propus	
1	11	3.25	2.97	2.95	99.4
2	15	3.60	3.28	3.35	102.0
3	15	4.06	3.86	3.88	100.4
4	16	4.47	4.23	4.29	101.5
5	12	4.79	4.59	4.67	101.7
6	11	5.24	5.21	5.22	100.2
7	10	5.65	5.71	5.79	101.1
8	16	6.04	6.11	6.195	101.1
9	11	6.47	6.43	6.56	102.1
10	10	7.21	7.45	7.58	101.7
11	6	8.00	8.28	8.35	100.9
12	3	8.63	9.00	9.10	101.1
13	4	9.40	9.675	9.775	101.0
14	3	11.13	12.40	12.53	101.0
15	2	12.55	14.40	14.55	101.0
16	2	14.70	16.55	16.90	102.1

TABLE VIII

LENGTH OF PROPUS OF RIGHT CHELA REGENERATED DURING ONE INSTAR IN
Portunus sayi, ARRANGED BY CARAPACE-LENGTH CLASSES. FROM TABLE
10 OF ZELENY (1908)

Class	No. of specs.	Mean length (mm) of	
		Carapace	Regenerated chelar propus
1	5	3.98	3.06
2	11	4.45	3.28
3	12	4.83	3.71
4	9	5.50	3.90
5	6	6.08	4.90
6	6	6.43	4.92
7	12	7.10	5.72
8	6	7.87	6.50
9	10	8.52	7.18
10	6	9.50	8.12
11	7	10.66	9.09
12	1	14.50	14.40

cient of growth-partition found for other organisms, and,
further, that the second of Zeleny's conclusions cited is
not strictly true, as the coefficient of growth-partition is
slightly higher for regenerating than for normal chelae.

SUMMARY

A number of numerical data found in the literature have been analysed in relation to the idea of differential growth-ratios and growth-gradients. The following chief results have been obtained.

(A) *Crustacean abdomens*

(1) The female abdomen of *Pinnotheres* (data of Atkins) has, both for breadth and length, a steep growth-gradient with growth-center in the terminal segment. The growth-gradient for length is at a lower absolute level than that for breadth, but is steeper. The size at which this rapid differential growth begins appears to vary by at least one instar.

(2) The female abdomen of *Telmessus* (data of Sasaki) has a much flatter growth-gradient (only determined for breadth) with growth-center in the subterminal (6th) segment. The corresponding growth-gradient for the male abdomen is still flatter, with growth-center between the 4th and 5th segments.

(B) *Negatively heterogonic Crustacean limbs*

The Racing Crab *Ocypoda* (data of Cott) shows a negative heterogony of its walking limbs, whereas its chela is isogonic. In positively heterogonic Crustacean walking legs so far investigated, the center of maximum growth is in the merus. Here, the merus is the center of *minimum* growth. The growth-gradient for all the walking legs is of similar, rather complex, form, quite different from that for the chela, which resembles that for the immature chela of *Eupagurus*.

(C) *Negative growth-gradients in the limbs of sheep*

This association between negative heterogony and a center of minimum growth is also seen in the limbs of sheep (data of Hammond). Here, however, the growth-gradient (which includes the limb-girdles) is of simpler type. The form of the gradient alters markedly about the time of birth.

(D) *Growth-profile in young herring*

During metamorphosis, the herring (data of Ford) shows radical differences in the growth-intensity of dif-

ferent regions.   Dorsal and ventral parts of the same region of the body may show different growth-coefficients.

Quantitative evaluation of these has permitted the construction of an approximate "growth-profile" for the whole organism.

(E) Analysis of Zeleny's data shows that the right chelar propus length of *Portunus sayi* has a nearly constant differential growth-ratio (coefficient of growth-partition) of about 1.15 relative to carapace-length, and that the coefficient of growth-partition for regenerating chelar propus after 1 moult-period is slightly higher, *viz.*, about 1.20.   Thus larger chelae regenerate a slightly greater proportion of propus-length during one moult period than do smaller chelae.

LITERATURED CITED

Atkins, D.
    1926. "The Moulting Stages of the Pea-Crab, (*Pinnotheres pisum*),"
        J. Mar. Biol. Assn. 14: 475–492.
Bush, S. F.
    1930. "Asymmetry and Relative Growth of Parts in the Two Sexes of
        the Hermit-Crab, *Eupagurus prideauxi*," *Arch. f. Entw.-*
        Mech. 123: 39–79.
Cott, H. B.
    1929. "Observations on the Natural History of the Racing Crab
        Ocypoda ceratophthalma, from Beira," *Proc. Zool. Soc.* 1929,
        755–765.
Ford, E.
    1930. "Herring Investigations at Plymouth. VIII.   The Transition
        from Larva to Adolescent," *J. Mar. Biol. Assn.* 16: 723–752.
Hammond, J.
    1927. *Verh. V. Internat. Kongr. Vererbungswiss.*   Berlin, Vol. 2.
    1929. "Probleme der Fleischerzeugung," *Zts. f. Züchtungsk.* 4: 543.
Huxley, J. S.
    1927. "Further Work on Heterogonic Growth," *Biol. Zentralbl.* 47:
        151–163.
Sasaki, K.
    1928. "On the Growth of *Telmessus cheiragonus* (Tilesius)," *Sci. Rep.*
        Tohoku Imp. Univ. 4th s. Biol.) 3: 805–819.
Shaw, M. E.
    1928. "A Contribution to the Study of Relative Growth of Parts in
        Inachus dorsettensis," *Brit. J. Exper. Biol.* 6: 145–160.
Smith, G. W.
    1905. *Fauna und Flora des Golfes von Neapel*, Vol. 29 (Rhizocephalus).
Zeleny, C.
    1908. "Some Internal Factors Concerned with the Regeneration of
        the Gulfweed Crab (*Portunus sayi*)."   Carnegie Publ. 103.
        (Papers from the Tortugas Lab. Vol. 2), 105.

INHERITANCE IN *NICOTIANA TABACUM*
XI. THE FLUTED ASSEMBLAGE

PROFESSOR ROY ELWOOD CLAUSEN

UNIVERSITY OF CALIFORNIA

THE studies herein reported in outline deal with a series of derivative forms of *Nicotiana tabacum* var. *purpurea* (U.C.B.G. 25/06, Setchell, 1912) which have arisen spontaneously under circumstances about to be described. Completion of the investigations will require some further years; but it seems justifiable to present a preliminary account of them at this time, in advance of the presentation of the details, in order that the relationship of the various forms to one another may be set forth in an intelligible fashion.

N. tabacum var. *purpurea* has been chosen arbitrarily as the base of reference in our experiments with this species. Maintained by self-fertilization since 1906, it has no doubt long since become completely homozygous, if this system of propagation can achieve such a result. Its high degree of uniformity makes it possible to detect and study minor departures from type which otherwise might pass unnoticed. Despite its prevailing constancy, it does produce occasionally variants, which, insofar as we have studied them, appear to be due mainly to irregularities in distribution of chromosomes. They probably arise more often as a consequence of non-conjunction than of non-disjunction; at any rate PMC figures at IM sometimes exhibit $23_{II} + 2_I$ chromosomes instead of the normal 24_{II}. One of the most frequently detected of the variants is the monosomic, fluted; and the present paper is confined to an account of new derivative forms which have appeared during our studies of it. Judging from these investigations, variation in chromosome number may be the starting-point for a variety of other kinds of genetic modification.

Fluted: Studies of fluted have already been reported in some detail (Clausen and Goodspeed, 1926). Phenotypically fluted is characterized by numerous minor departures from the normal type; shorter, more intensely colored flowers with a peculiarly fluted limb, a more compact inflorescence, slightly telescoped growth, somewhat smaller and rounder leaves more erectly borne on the stem, and other less pronounced differences, constituting together a readily recognizable complex of characters. PMC figures at IM uniformly exhibit $23_{II} + 1_I$ chromosomes; the single being a large chromosome, characteristically somewhat constricted in the middle. This single chromosome we shall call the F-chromosome; fluted, then, is haplo-F. Fluted ♀ x normal ♂ produces about 40 per cent. normal and 60 per cent. fluted offspring; but the reciprocal cross gives about 98 per cent. normal and only 2 per cent. fluted offspring. Evidently 23-chromosome gametes are present in the female series somewhat in excess of the 50 per cent. expectation, probably as a consequence of lagging of the univalent during meiosis; and 23-chromosome pollen grains are at a marked disadvantage in competition with normal 24-chromosome pollen grains. Fluted is ordinarily maintained by crosses of fluted ♀ x normal ♂ in order to insure genetic identity of the two types; and in our experience, under this system of propagation, the offspring has always consisted exclusively of normal and fluted individuals.

Fluted x self: When fluted is selfed the progeny usually consists of normal and fluted plants in about the same proportions as from fluted ♀ x normal ♂. Such populations have been grown on a number of occasions with the object of obtaining the 23_{II}, nullo-F form corresponding to fluted; but it has never been secured and it is probably an inviable type. However, two new derivatives, coral and mammoth, have been obtained from such populations, in both instances as single variant individuals from which constant stocks have been established.

Coral, named from the distinctive coral or salmon pink color of its flowers as contrasted with the carmine of normal and fluted, is a striking type. With its semi-dwarf habit of growth and small, light green leaves, it exhibits as marked contrasting features to normal vegetatively as in flower color. There are two forms of it, normal coral and fluted coral, which exhibit phenotypic differences parallel to those between normal and fluted. PMC preparations of normal coral show 24_{II} chromosomes, apparently indistinguishable from the normal chromosomal garniture; and those of fluted coral have $23_{II} + 1_I$, the single being of the same type as that in unmodified fluted (Clausen, 1930).

Mammoth is apparently the same character as is represented in such commercial varieties as Maryland Mammoth. It is characterized by slow but long-continued growth, leading to the production of tall, unbranched plants with numerous leaves. As it approaches maturity it branches in candlelabrum fashion at the top; but with us it ordinarily does not bloom until very late in the season, if at all. Grown in the greenhouse from fall sowings during the winter, however, it blooms only slightly later than normal plants. Evidently our type has the same response to length of day noted by Garner and Allard (1920) for the commercial mammoth. The blossoms are practically identical with those of normal both in size and color. Like coral, mammoth may exist in the normal and fluted conditions with 24_{II} and $23_{II} + 1_I$ chromosomes, respectively.

Both of these types are recessive to normal, the heterozygote in each case exhibiting only a slight departure towards intermediacy. In both instances, moreover, the new form has arisen by modification of the F-chromosome, as is shown by the following results:

fluted ♀ × coral ♂ → F_1 normal + fluted coral

fluted ♀ × mammoth ♂ → F_1 normal + fluted mammoth

Crosses of coral x mammoth produce normal F_1, indicating that the modifications involve different elements of the F-chromosomes. Without for the present committing ourselves to the hypothesis of factor mutation as responsible for their origin, we may assign the formula F-co F-co to coral and F-mm F-mm to mammoth.

Coral x normal: As we have just stated, coral x normal produces normal F_1. The F_2 progenies in the main closely approach the expected ratio of 3 normal: 1 coral; but they also contain scattering representatives of other types of which the following appear regularly:

$$\text{large lax} \quad 24_{II} + 1_I,$$
$$\text{fluted} \quad 23_{II} + 1_I,$$
$$\text{fluted coral} \quad 23_{II} + 1_I.$$

The large lax type has flowers considerably larger than normal, with a wider throat and somewhat lighter color; the plants are taller, leaves longer and narrower and with reduced auricles, internodes longer, and the growth is characteristically lax, so that the plants often have crooked stems. In general, the large lax form exhibits the opposite kind of departure from the normal type as compared with that seen in fluted. Cytological examination reveals the presence of an extra chromosome, hence large lax is trisomic. In general features it closely resembles the trisomic which we have previously described under the designation enlarged (Clausen and Goodspeed, 1924).

The other two types, fluted and fluted coral, are identical, phenotypically and cytologically, with the forms previously described under these names.

The explanation for the appearance of these exceptional individuals in F_2 appears clearly to be afforded by cytological examination of F_1 plants, PMC preparations of which frequently exhibit $23_{II} + 2_I$ chromosomes instead of 24_{II}. It would appear that non-conjunction of the F-chromosomes occurs frequently in normal plants heterozygous for coral, as a consequence of which some

nullo-F and diplo-F gametes are produced. These account for the production of fluted and fluted coral plants in F_2 and also for large lax if this type is triplo-F. If so, large lax must be genetically distinct from the trisomic enlarged which has been shown not to involve the F-chromosome (Clausen and Goodspeed, 1926).

Besides the variant types which have just been mentioned and which appear to be due to non-conjunction of the F-chromosomes, a number of others have appeared in some F_2 progenies. Of these, we consider here only one, a type called pale sterile, which has appeared in approximately one fourth of the individuals of some, but not all, F_2 progenies. Pale sterile is somewhat paler as to flower color than normal and differs from it slightly in some other respects. Its characteristic feature is its pronounced sterility; for it produces practically no functional pollen, and judging from the results of pale sterile ♀ x normal ♂ crosses, only about 10 per cent. of its ovules are functional. Examination of PMC figures of pale sterile reveals extensive failure of conjugation as a consequence of which the figures have the appearance of those of interspecific hybrids, except that there appears to be no regular number of bivalents and univalents. Evidently pale sterile is an asynaptic form comparable to the one which Beadle (1930) has described in maize. Pale sterile appears to segregate independently of coral, hence, it does not appear to be due to a modification of the F-chromosome. The type has also been observed to segregate in some coral populations, hence it has probably been introduced with the coral in these crosses. It has not, however, been observed in other lines of *N. tabacum*.

Mammoth x normal: F_1 mammoth x normal is normal and F_2 progenies consist approximately of 3 normal: 1 mammoth. Apparently, however, the same sort of irregularities arise as in the crosses of coral x normal; but the populations have not been so satisfactorily class-

ified on account of the failure of mammoth plants to blossom during the normal growing season. As with coral, occasional fluted and large lax plants have been observed in F_2 progenies. Inasmuch as the mammoth plants did not bloom, it could not be determined satisfactorily whether or not any of them were fluted; but some of them certainly exhibited the peculiar vegetative characters of that type.

Coral x mammoth: As stated above coral x mammoth gives normal F_1. The cross was made for the express purpose of determining the linkage relations of coral and mammoth, inasmuch as the crosses with fluted had shown them both to be due to modification of the F-chromosome. In the absence of a coral mammoth stock, the customary backcross could not be made. As a substitute crosses were made of fluted ♀ x F_1 coral x mammoth ♂ which should produce fluted offspring of various classes corresponding to the types of gametes produced by F_1 coral x mammoth. The results were as follows:

114 normal
52 fluted coral
58 fluted mammoth
2 fluted
0 fluted coral mammoth

Only the fluted classes are of significance for the problem at issue; fluted coral and fluted mammoth representing the parental combinations and fluted and fluted coral mammoth the recombinations. The figures presented might be interpreted to indicate about two per cent. of crossing-over between coral and mammoth. However, it is to be noted that no fluted coral mammoth plants were obtained and the two fluted plants may have arisen from 23-chromosome pollen grains produced as a result of nonconjunction of the F-chromosomes in F_1 coral x mammoth. Further evidence in support of this interpretation was afforded by the presence of seven large lax plants of the type previously described, which are in-

cluded among the normal plants in the above tabulation. If these plants are triplo-F, as has been suggested above, they should have the constitution, F F-co F-mm; *i.e.,* their three F-chromosomes should be different.

However, included among the fluted coral plants in the above tabulation were three plants which were normal coral; and, although the same difficulties were experienced in dealing with the mammoth plants as in other populations, a few of them appeared from the vegetative characters to be normal rather than fluted. Apparently equational non-disjunction must be responsible for their appearance; thus we reach the conclusion that non-conjunction or the unpaired condition of a chromosome may predispose to consequent equational non-disjunction. In view of this fact, it is of course possible for the large lax plants to possess a variety of constitutions, instead of the one stated above.

Fluted x coral: As was stated above, fluted ♀ x coral ♂ gives normal and fluted coral offspring in F_1. In addition to these expected offspring, however, four separate instances of origin of carmine-coral variegation have been noted. These variegated plants had a carmine continuous and a coral disperse phase, in which respect they exhibit the opposite condition from that shown by the classical instances of variegation in which the recessive is the continuous and the dominant the disperse phase. The four instances of variegation were all different phenotypically, both as respects the type of variegation and the vegetative features of the plant. The first instance, carmine-coral-I, had vegetative features almost identical with coral; but the flowers, although of the same size as those of coral, are carmine; flecked, flaked and striped with coral. Somatic reversion to self-coral occurs frequently; to self-carmine rarely, if at all.

The other three instances arose from a cross of fluted mammoth ♀ x fluted coral ♂ and have only been seen in single plants as yet. However, carmine-coral-II had

vegetative characters close to normal and a coarser type of variegation. Carmine-coral-III also had vegetative characters closely approaching those of normal plants and flowers nearly normal in size; but the coral flecks were usually sparse and small. Carmine-coral-IV had somewhat larger flowers than those of carmine-coral-I; but had a close resemblance to it in vegetative features and type of variegation.

In carmine-coral-I it has been shown (Clausen, 1930) that the varigation is associated with the presence of a small, fragmentary chromosome, which is interpreted, in view of the parentage and the phenotypic effects, as a fragment of the F-chromosome bearing the carmine flower color complex, represented by f-Co. In terms of this notation normal carmine-coral is F-co F-co f-Co and fluted carmine-coral, F-co f-Co. The variegation appears to depend on sporadic loss of the fragment during development.

Carmine-coral x coral: Carmine-coral-I has been studied genetically in some detail through employment of a variety of crosses of normal and fluted coral with normal and fluted carmine-coral and through selfing of the two latter types. These results will be reported fully in the next following paper in this series. In the main they show a low and erratic ratio of transmission of variegation, amounting on the average to about fourteen per cent. both in the female and male series of gametes; and not infrequently individual carmine-coral flowers fail completely to transmit variegation. Doubtless this last behavior depends upon early elimination of the fragment; but the results are not consistent with the characteristic features of particular flowers, owing to the cell layer relations involved in flower color production (Clausen and Goodspeed, 1923).

Of outstanding significance, however, is the occasional production of self-carmine plants in progenies from variegated parents. Seven separate instances of this phe-

nomenon are on record; its ratio of occurrence thus far
is about three for every hundred variegated plants. Just
how this reversion to self-carmine has been accomplished
has not yet been determined, but one possibility may ap-
parently be eliminated at the outset. Four of the re-
corded instances arose in crosses of fluted carmine-coral
♀ x coral ♂, and all four of them were fluted self-carmine
plants. They could not have arisen by reattachment of
the fragment to the F-chromosome for these plants re-
ceived their only F-chromosome from their paternal
parent and the carmine came in from the maternal side.

Pale sterile x normal: Progenies grown from pale
sterile ♀ x normal ♂ are highly variable, but the plants,
with occasional exceptions, do not present any very ex-
treme departures from the normal type. Preliminary
cytological examinations of individual plants in such a
progeny disclosed a variation in chromosome number
such as might be predicted from the irregular distribu-
tion of chromosomes observed in pale sterile. Accurate
counts were extremely difficult to make; but apparently
the offspring were all modified diploids; *i.e.*, they had 24_{II}
chromosomes plus and minus, or in other words the
plants were trisomic for some and monosomic for other
chromosomes. No triploids were detected among them,
in which respect, as well as in the production of mono-
somics, the results differ somewhat from those described
by Beadle (1930) for his asynaptic maize. Employment
of pale sterile, therefore, represents an addition to the
already numerous methods of obtaining plants with
variant chromosome numbers in *N. tabacum,* but it ap-
pears to be a particularly excellent one, for apparently
one may obtain the entire series of primary monosomic
and trisomic types from it.

DISCUSSION

With the possible exception of pale sterile, the fore-
going series of forms all appear to involve variation

either in the number of F-chromosomes or in their constitution, for which reason we call them the fluted assemblage. Those which arise by variation in the number of F-chromosomes require no further comment; but those which are due to some internal modification of the chromosome present some rather interesting problems. The mutational sequence in these instances was of the form, normal → haplo-F → haplo-modified-F; and in view of the fact that the modified forms arose from fluted x self but not from fluted x normal, they appear to be due to some special form of modification to which the chromosome is occasionally subject when in the univalent or unpaired condition.

In mammoth we deal with a character the origin of which has been reported independently by a large number of investigators, and which has been utilized extensively in commercial practice. In general, genetic investigations with it support the conclusion reached by Allard (1919) that it is a simple recessive to normal; and the origin of the character has often been cited as a typical example of factor mutation. But it is at least not the usual thing for factor mutations to disturb the conjugation of the chromosomes in which they are located; and as for the evidence from genetic studies, it may be dismissed as inadequate, for any genetic alteration which behaves as a unit and does not impair the vitality or viability of the gametes and zygotes will give results superficially identical with those of simple factor differences. Moreover, various puzzling phenomena have been reported in connection with previous studies of mammoth which indicate that the same type of irregularity in chromosome distribution detected in our experiments has also occurred in them. Thus both Lodewijks (1911) and Honing (1914) reported the occurrence of an intermediate giant type, probably triplo-F, which segregated for normal. It may be recalled also that Hayes and Beinhart (1914) found that a few mutant mammoths

appeared in the progeny of normal plants which were apparently not heterozygous for the character, and felt impelled on the basis of this fact to conclude that the mutation must have occurred after fertilization. If, as is almost certainly the case, undetected fluted plants were present in their plantings, the mutational sequence, normal → fluted → fluted mammoth, may have been in operation in their instances as in ours. At any rate this explanation avoids the obvious difficulty of their interpretation, since the presence of a single F-chromosome in fluted makes it possible for recessive mutations in this chromosome to manifest their occurrence immediately.

More cogent evidence is afforded from the study of coral and carmine-coral variegation. In carmine-coral plants a fragment is present which bears a factor complex capable of restoring the normal carmine color, but leaving the no less marked vegetative features of coral only slightly altered. When this complex becomes stable, as in reversionary self-carmine, a type is produced identical as to flower color with normal but exhibiting an only slightly more robust growth than coral. The fragment, therefore, has a portion of the genetic material necessary to restore coral to the normal condition, but not all; or to put the matter in another way, the coral flower color and the vegetative features characteristic of the type are due to alteration of different germinal elements. Coral, therefore, depends upon some genetic modification more extensive than simple factor mutation.

The occurrence of fragmentation, as evidenced in the origin of carmine-coral variegation, suggests that a similar phenomenon on a less extensive scale may have been responsible for the origin of coral and mammoth. PMC preparations of fluted not infrequently exhibit the univalent in IA stretched out from one chromosome group to the other, suggesting that it may be torn in pieces simply as a consequence of its univalent condition. In view of the fact that nullo-F gametes are functional,

there seems to be no reason why a gamete containing any part of the F-chromosome should not also be functional, and the question as to whether the process described would give rise to new genetic types simply hinges on the possibility that zygotes arising from such gametes may be viable. In effect, this process would give rise to deficiencies, and in general those which have been studied heretofore, mainly in Drosophila, have been lethal when homozygous. But *N. tabacum* is probably a polyploid species (Clausen, 1928) and under these circumstances it would appear probable that deficiencies would have a less serious effect than in species such as those of Drosophila. On the cytological side, however, both in coral and mammoth, if the alteration is of the nature of deficiency, it is insufficient in extent to be recognizable cytologically. Since the modified F-chromosome is present in the univalent condition in fluted coral and fluted mammoth, it may be identified with certainty in meiotic divisions. Comparisons of the univalent chromosomes of these types with that of fluted seem to indicate that they are identical morphologically.

As an alternative to simple deficiency, the possibility might be considered that they represent a modification of the type postulated by Belling and Blakeslee (1924) to account for those secondary trisomics of Datura which contain an extra chromosome modified genetically but unaltered in size. Their suggestion is equivalent to a deficiency of one half of the chromosome and a duplication of the other half, and they have advocated an hypothesis of reversed crossing-over to account for it. The hypothesis at least is inapplicable in the present instances, for the modification occurred in individuals which had only one F-chromosome. Possibly a similar condition may arise by fragmentation of a single chromosome, inasmuch as a loss of part of the chromosome and a duplication of the remaining portion through the medium of the equational split might occur simultaneously.

Whatever the precise nature of the chromosomal modification responsible for the production of coral and mammoth, the results afford a demonstration of the possibility of obtaining new and distinctive races of *N. tabacum* by substitution of modified chromosomes, genetically altered in some way other than by factor mutation, for normal ones. In most instances when genetic modification has gone beyond the limits of factor mutation, barring those in which the elements are all present but with an altered organization, no complete replacement of the normal chromosome has been possible. Thus the secondary modifications of Datura (Blakeslee, 1924, 1928) are apparently obtainable only in the trisomic condition in association with two unmodified chromosomes. It is, of course, probable that, as in the case of mammoth, some instances hitherto ascribed to factor mutation or to some other kind of alteration, actually belong in the same category.

The speltoid and related forms of wheat studied genetically by Nilsson-Ehle (1921, 1927) and Linhard, (1922, 1927) and cytologically by Winge (1924), Huskins (1928b) and others and the fatuoid series in oats (Huskins 1927, 1928a) present many features in common with the fluted assemblage; but the details are so complex and the relation between the cytological and genetical findings so dubious, that it seems of doubtful value to attempt a discussion of the various explanations which have been offered for them, particularly as the whole situation has recently been reviewed by Watkins (1930). It may, however, be possible that some of the 42-chromosome fatuoid and speltoid derivatives owe their origin to secondary genetic modification arising as a consequence of a previous monosomic or trisomic condition rather than to the replacement of chromosomes of one set by corresponding chromosomes from another or to crossing-over between corresponding chromosomes of different sets. Possibly the phenomena here described occur only in polyploid

species, but in view of the great number of such species in plants, this restriction would not greatly reduce their importance.

Summary

1. By loss of an F-chromosome, probably as a consequence of occasional non-conjunction, *N. tabacum* var. *purpurea* produces the monosomic, fluted.

2. When selfed, fluted has produced as further variants the distinctive recessive types, coral and mammoth, both of which have been established in pure lines.

3. From crosses with fluted, both coral and mammoth have been shown to be due to secondary modification of the F-chromosome.

4. Coral and mammoth, when crossed with normal or with each other, produce hybrids of the normal type, which, however, exhibit frequent non-conjunction of the F-chromosomes.

5. By reason of this non-conjunction, F_2 and other derivative populations from these crosses contain some haplo-F and triplo-F individuals in addition to the expected classes of offspring.

6. Hybrids of coral with mammoth apparently exhibit no crossing-over between the two modified F-chromosomes.

7. Some of the F_2 populations of coral $\times$ normal segregated for a recessive pale sterile type, which owes its sterility to extensive non-conjunction of the chromosomes in meiosis.

8. Crosses of pale sterile $\female \times$ normal $\male$ produce a variable offspring consisting of numerous monosomic, trisomic and other more complex types of chromosomal variants.

9. Crosses of fluted $\female \times$ coral $\male$ and fluted mammoth $\female \times$ coral $\male$ have produced four instances of carmine-coral variegation, one of which has been shown to arise from fragmentation of the F-chromosome.

10. Carmine-coral variegated plants produce occasional reversionary self-carmine variants, which are identical in flower color but not in vegetative features with normal.

11. Evidence from carmine-coral variegation and reversionary self-carmine indicate that the vegetative features of coral and its distinctive flower color must be due to alteration of different components of the F-chromosome; hence coral must represent some modification of the F-chromosome more extensive than simple factor mutation.

12. Evidently the F-chromosome in the univalent condition is subject to some special forms of genetic modification which occur rarely, if at all, in normal material.

LITERATURE CITED

Allard, H. A.
    1919. "Gigantism in *Nicotiana tabacum* and its Alternative Inheritance," Amer. Nat., 53: 218–233.
Belling, J., and Blakeslee, A. F.
    1924. "The Configuration and Sizes of the Chromosomes in the Trivalents of 25-Chromosome Daturas," *Proc. Nat. Acad. Sci.*, 10: 116–120.
Blakeslee, A. F.
    1924. "Distinction between Primary and Secondary Chromosomal Mutants in Datura," *Proc. Nat. Acad. Sci.*, 10: 109–116.
    1928. "Genetics of Datura," *Verh. V. intern. Kong. Vererb.*, 1: 117–130.
Clausen, R. E.
    1928. "Interspecific Hybridization and the Origin of Species in Nicotiana," *Verh. V. intern. Kong. Vererb.*, 1: 547–553.
    1930. "Inheritance in *Nicotiana tabacum*. X. Carmine-coral Variegation," *Cytologia*, 1: 358–368.
Clausen, R. E., and Goodspeed, T. H.
    1923. "Inheritance in *Nicotiana tabacum*. III. The Occurrence of Two Natural Periclinal Chimeras," *Genetics*, 8: 97–105.
    1924. "Inheritance in *Nicotiana tabacum*. IV. The Trisomic Character, Enlarged," *Genetics*, 9: 181–197.
    1926. "Inheritance in *Nicotiana tabacum*. VII. The Monosomic Character, Fluted," *Univ. Calif. Publ. Bot.*, 11: 61–82.
Garner, W. W., and Allard, H. A.
    1920. "Effect of the Relative Length of Day and Night and Other Factors of the Environment on Growth and Reproduction in Plants," *J. Agr. Res.*, 18: 553–606.

Hayes, H. K., and Beinhart, E. G.
    1914. "Mutation in Tobacco," *Science,* 39: 34–35.
Honing, J. A.
    1914. "Deli-Tabak, een Mengel van Rassen die in Bladbreedte en
        Aantal Bladeren Verschillen," *Med. Proef. Medan,* 8: 155–183.
Huskins, C. L.
    1927. "On the Genetics and Cytology of Fatuoid or False Wild Oats,"
        J. Genetics, 18: 315–364.
    1928a. "Genetical and Cytological Studies of Fatuoid Oats and Speltoid
        Wheats," *Verh. V. intern. Kong. Vererb.,* 2: 907–916.
    1928b. "On the Cytology of Speltoid Wheats in Relation to Their
        Origin and Genetic Behavior," *J. Genetics,* 20: 103–122.
Linhard, E.
    1922. "Zur Genetik des Weizens," *Hereditas,* 3: 1–90.
    1923. "Fortgesetzte Untersuchungen über Speltoidmutationen," *He-
        reditas,* 4: 206–220.
    1927. "Über Ährendichte und Spaltungsmodi der Speltoidheterozy-
        goten," *Kong. Vet. og Land. Aarskrift,* 1927: 1–37.
Lodewijks, J. A.
    1911. "Erblichkeitsversuche mit Tabak," *Zeit. ind. Abstamm. u.
        Vererbl.,* 5: 139–172.
Nilsson-Ehle, H.
    1921. "Über mutmassliche partielle Heterogametie bei den Speltoid
        Mutationen des Weizens," *Hereditas,* 2: 25–76.
    1927. "Das Verhalten partieller Speltoidmutationen bei Kreuzung
        untereinander," *Hereditas,* 9: 369–379.
Setchell, W. A.
    1912. "Studies in *Nicotiana.* I.," *Univ. Calif. Publ. Bot.,* 5: 1–86.
Watkins, A. E.
    1930. "The Wheat Species: a Critique," *J. Genetics,* 23: 173–263.
Winge, Ø.
    1924. "Zytologische Untersuchungen über Speltoide und andere mu-
        tantenähnliche Aberranten beim Weizen," *Hereditas,* 5: 241–
        286.

STUDIES IN HUMAN INHERITANCE. V.[1]

MULTIPLE ALLELOMORPHISM AS OPPOSED TO LINKAGE IN BLOOD GROUP HEREDITY

PROFESSOR LAURENCE H. SNYDER

DEPARTMENT OF ZOOLOGY, OHIO STATE UNIVERSITY

THE inheritance of the human blood groups has been shown to be dependent upon a set of triple allelomorphs (Bernstein, 1925, Snyder, 1926, 1929, *et al.*). Based on this interpretation, laws relating to legal and clinical medicine, and to anthropology, have been formulated (Snyder, 1927, 1929, 1930). The evidence for the triple allelomorph hypothesis was considered adequate, and the question of the mode of inheritance of the blood groups was, I thought, settled. Former suggestions of hypotheses, involving, respectively, two pairs of independent factors and two pairs of completely linked factors, had been shown to be untenable.

In 1928, however, a short note appeared by K. H. Bauer, suggesting the assumption of two pairs of partially linked factors, instead of triple allelomorphs, as the basis for the inheritance of the blood groups. This hypothesis was said by its proposer to explain the exceptions which had been observed by various workers in the offspring of certain crosses. Bauer assumed 11 per cent. cross-over between the two linked factors.

This hypothesis, however, is genetically as untenable as the old hypothesis of independent assortment. In my monograph on the blood groups (Snyder, 1929), I mentioned briefly the linkage hypothesis, stating that it could not adequately explain the observed facts, and giving concise specific reasons for my statement.

In spite of this, however, in two text-books which have just appeared, Bauer's hypothesis of linked factors is accepted.

Gates, in his "Heredity in Man," p. 196, after review-
ing the blood group data, puts forward Bauer's new
hypothesis, saying that it ". . . appears to resolve the
difficulties in the two previous theories." He also states
that on this basis "the results appear to compare fav-
orably with expectation." Likewise Castle, in the fourth
revised edition of his "Genetics and Eugenics," just off
the press, says, p. 372, ". . . On the whole, the recorded
observations accord best with the second interpretation,
which regards A and B as mutations in different genes
borne in the same chromosome, and so with only oc-
casional crossing over between them."

The acceptance of the hypothesis of linked factors in
two such widely read texts as those cited makes it neces-
sary for me to refute specifically the arguments advanced
in favor of this explanation of the heredity of the blood
groups.

Bauer's reason for advancing the linked factor hy-
pothesis was that on the basis of triple allelomorphs
some exceptional results are obtained. Specifically, in
crosses of O and AB, only children of groups A and B
should be obtained, whereas occasional children of groups
O and AB have been recorded from such matings. The
hypothesis of linked factors purports to explain these.

There are two points to be developed in refuting this
claim. First, it is not at all certain that exceptions really
exist. Second, if some exceptions to the hypothesis of
triple allelomorphs do exist they can not be explained by
assuming linkage. A possible genetic explanation for
these, if they really exist, will be mentioned below.

To consider these two points, then, one at a time, let
us examine the recorded exceptions. On the basis of
triple allelomorphs, the genotypes of the four blood
groups are as follows:

Group O – OO
Group A – AA, AO
Group B – BB, BO
Group AB – AB

Thus, crosses of group O with AB should give only children of groups A and B, as follows:

$$OO \times AB$$
$$AO, BO$$

Actually, some children of groups O and AB have been recorded from such crosses. However, the vast majority of such exceptions were recorded in the early years of blood grouping (1910–1925), before the technique was standardized, and before anything was known of the triple allelomorph hypothesis. Since 1925 exceedingly few exceptional cases have been recorded. This is shown in Table I.

TABLE I

OFFSPRING OF CROSSES OF GROUPS O AND AB

	Numbers in group				Per cent. Exceptions
	O	A	B	AB	
(1) Before hypothesis of triple allelomorphs	31	94	61	26	36.7
(2) Since hypothesis of triple allelomorphs	14	405	400	7	
(3) With known errors removed from (2)	7	401	399	5	1.5

It may be seen from this table that previous to the triple allelomorph hypothesis there were 36.7 per cent. exceptions to the rule as based on triple allelomorphs. Since this hypothesis was put forward, however, only 1.5 per cent. exceptions have been recorded, although the total number examined is four times as large. The second line in Table I represents all the recorded exceptions, while the third line gives those which remain after removing the known errors from the second line. For example, of the 14 exceptional Group O children recorded since the triple allelomorph hypothesis, two were known to be illegitimate (Snyder, 1, Furnhata, 1) and four were wrongly included because of the mixing of two families of the same name (Kliewe and Nagel). Of the

seven exceptional group AB children, one was known to be illegitimate (Thomsen), and one was again the case of the confusion of two families of the same name (Kliewe and Nagel). With these removed, the percentage of exceptions is only 1.5, and this in spite of the fact that several investigators have concentrated specifically on this type of mating with the express purpose of looking for exceptions. The small remaining per cent. may be explained on the basis of illegitimacy, mistakes in technique, adoptions, mixing of babies in hospitals, or on the basis of non-disjunction, as will be shown later.

My own studies, involving more than 500 families, more than 25 of which are of O with AB, do not give any exceptions to the hypothesis of triple allelomorphs, except one case, which was on investigation shown to be a case of illegitimacy (Snyder, 1929). Personally, I am convinced that illegitimacy and the other possibilities just mentioned will fully account for any exceptions recorded.

Matings of groups O and AB are hard to obtain, since group AB is so rare. However, by taking the blood groups of mothers and children in maternity hospitals the problem may be indirectly attacked. Twelve investigators have done this, recording a total of 371 mothers and 371 children. The results are shown in Table II.

TABLE II
OFFSPRING OF GROUP AB MOTHERS

Number of families	Number in group			
	O	A	B	AB
371	2	196	150	175

It will be seen that the exceptions in this case are the group O children, since group AB children are possible inasmuch as the fathers could be of any of the four groups. Illegitimacy can not explain these, but mistakes in technique, adoptions, mixing of babies, etc., could. Of the 12 investigators, some of whom studied as many as a hundred cases, only one found exceptions. This worker

studied only seven families altogether, and recorded two exceptions.

The exceptions to the law of triple allelomorphs, then, are certainly not very numerous. Assuming, however, that some actual exceptions do occur, would they be explained by assuming linkage of the two factors concerned? In glancing at the last line of Table I, it would seem offhand that the second and third columns might represent non-crossovers, and the first and fourth might represent crossovers. Bauer so interpreted these columns, although he used the total results, and did not separate them into those recorded before the triple allelomorph hypothesis and those recorded afterwards.

Castle and Gates both interpreted the four columns as crossovers and non-crossovers. All three of these workers, however, have apparently completely lost sight of the fact that in a random-mating population the coupling and repulsion phases are not conveniently separated, as they are in cages of experimental animals, but that both phases will occur, *and in equal proportions,* even assuming only a very small per cent. of crossing over. Time is the only requisite. This would mean that the four columns in Table I should be approximately equal on the basis of linkage. Complete equality would never occur because of the presence of some single and double homozygotes among the group AB individuals. This point may profitably be examined in detail.

Let us, for the sake of argument, assume that the blood groups are, as Bauer suggests, inherited as linked factors. Then the genotypes of the four groups would be as follows:

        Group O   (ab)(ab)
        Group A   (Ab)(Ab), (Ab)(ab)
        Group B   (aB)(aB), (aB)(ab)
        Group AB (AB)(AB), (AB)(Ab), (AB)(aB),
                   (AB)(ab), (Ab)(aB)

Bauer and those who accept the linkage hypothesis apparently consider that group AB, resulting from the combinations of groups A and B, would be only of the

formula (Ab) (aB). If this were true, the results in Table I could properly be interpreted as crossovers and non-crossovers. The assumption is granted that the original phase was the repulsion phase, that is, that A and b were linked on one chromosome and a and B on the other. Thus the first individuals of group AB which appeared in the phylogeny of the blood groups were undoubtedly of the formula (Ab) (aB). This condition, however, would not prevail long, unless the factors were completely linked, and no further mutations took place.

If any crossing over at all occurred, the new combination (AB) would be formed. Since this would be as stable, once it was formed, as either of the original combinations (aB) or (Ab), it would only occasionally cross back when the opportunity was presented, that is, in combination with a chromosome carrying (ab). The new combination (AB) would thus tend to pile up, and it is this fact that makes the hypothesis of linked factors untenable. How extensively would the combination (AB) pile up? And how would this invalidate the hypothesis of linked factors? Let us examine the material on a frequency basis. Let p equal frequency of A, q equal the frequency of a, r equal the frequency of B, and s the frequency of b. Then $p + q$ equals 1 and $r + s$ equals 1.

Based purely on laws of chance recombination, the combination (AB) should reach a maximum frequency in the general population of pr, as follows:

	A	a
	p	q
B r	AB pr	aB qr
b s	Ab ps	ab qs

The question arises, Would linkage invalidate this? Would (AB) actually reach this frequency, or might it even exceed it?

The piling up of (AB) chromosomes will cease, and a balance between the four combination (AB), (Ab), (aB)

and (ab) will be struck, when the double heterozygotes of group AB consist of as many individuals of the coupling phase (AB) (ab) as of the repulsion phase (Ab) (aB), since these combinations are the only ones in which crossing over can change the factor relationships, and since crossing over in either of these phases results in the production of gametes of the other phase. If the union of the four kinds of gametes in the general population is represented by the usual checkerboard, and the gametic combinations entering into the various individuals are kept in parentheses, to indicate the linkage, the coupling and repulsion phases of group AB may be distinguished. It will be seen that as far as numbers of squares in the checkerboard are concerned, there result equal amounts of the coupling and repulsion phases. But this is without consideration of the frequencies of the various factors concerned. Would this alter the equality? If the letters p, q, r and s are now added to the checkerboard, it will be seen that using the frequency pr for AB, and the corresponding frequencies for the other gametes, the coupling and repulsion phases of the double heterozygotes of group AB will still be equal; that is, the number of individuals of (AB) (ab) will be equal to the number of individuals of (Ab) (aB), because each will occur with the frequency 2 pqrs, as follows:

	AB pr	Ab ps	aB qr	ab qs
AB pr	(AB)(AB) p^2r^2	(AB)(Ab) p^2rs	(AB)(aB) pqr^2	(AB)(ab) $pqrs$
Ab ps	(AB)(Ab) p^2rs	(Ab)(Ab) p^2s^2	(Ab)(aB) $pqrs$	(Ab)(ab) pqs^2
aB qr	(AB)(aB) pqr^2	(Ab)(aB) $pqrs$	(aB)(aB) q^2r^2	(aB)(ab) q^2rs
ab qs	(AB)(ab) $pqrs$	(Ab)(ab) pqs^2	(aB)(ab) q^2rs	(ab)(ab) q^2s^2

Thus, if linkage were concerned, the conditions for the maximum frequency pr of the combination (AB) would be met, quite independently of the frequencies of the

factors concerned or of the amount of crossing-over occurring, the only requirement being the elapsing of sufficient time. In the case of the human blood group this may be safely assumed. The frequencies of the factors concerned in linkage would thus be equivalent, after a balance is reached, to those in independent assortment.

We may then derive in the usual way from the above checkerboard the frequencies p, q, r and s in terms of the percentage of the four groups. These percentages would of course be known quantities in any particular race. This is done briefly as follows:

$$\text{Group O} = q^2 s^2$$
$$\text{Group A} = p^2 s^2 + 2\ pqs^2 = s^2(1-q^2)$$
$$\text{Group B} = q^2 r^2 + 2\ q^2 rs = q^2(1-s^2)$$
$$\text{Group AB} = p^2 r^2 + 2\ pqr^2 + 2\ p^2 rs + 4\ pqrs = (1-q^2)(1-s^2)$$

From the first three equations, p, q, r and s may be calculated in various ways. The most accurate way, as I have previously shown, is as follows:

$$q = \sqrt{\frac{O}{O+A}}$$
$$s = \sqrt{\frac{O}{O+B}}$$
$$p = 1 - q$$
$$r = 1 - s$$

From this it is easy to calculate the frequencies of the gametic combinations, since we have expressed these as pr, ps, qr and qs. By filling in these gametic frequencies in the above checkerboard, we may obtain the expected frequency of any genotype (and so of any phenotype) in any particular population. Using my percentage of the four groups among Americans, we find the frequencies p, q, r and s to be .3, .7, .1 and .9, respectively. The frequencies of (AB), (Ab), (aB) and (ab) are thus .03, .27, .07 and .63, respectively.

If we now consider crosses of AB × O, on the basis of linked factors, we find that the AB members of such crosses, even if close linkage occurred, would after all these years of crossing over consist of equal amounts of the coupling and repulsion phases (double heterozygotes), plus some single heterozygotes [(AB) (Ab),

(AB) (aB)], and some double homozygotes (AB) (AB). Among Americans, the double heterozygotes [(AB) (ab) and (Ab) (aB)] should each occur in 3.78 per cent. of the general population. The single heterozygotes (AB) (Ab) should occur in 1.62 per cent., the single heterozygote (AB) (aB) in 0.42 per cent., and the double homozygote (AB) (AB) in 0.09 per cent.

The above figures give us two separate bases for disproving the hypothesis of linked factors as an explanation of the heredity of the human blood groups. In the first place, if we add up the frequencies of the various genotypes of group AB, we obtain a total frequency of AB of 9.69 per cent. This is much larger than the observed per cent. (4.0), and brings us right back to the original statistical objection to the hypothesis of two factors, linked or not linked. This deficiency of group AB, in comparison with expectation, holds for all races studied, whereas on the triple allelomorph hypothesis the observed proportion agrees closely with the expected.

In the second place, it is seen by examining the expected results of crosses of O and AB on the basis of linkage that they are entirely contrary to the observed results.

From the figures given above we may determine the relative proportions of the various genotypes within the group AB. Using these figures, the expectation in crosses of groups O and AB among Americans is as follows:

Genotype of cross	Proportion of total AB	O	A	B	AB
(AB)(AB) × (ab)(ab)	.009	....	....	....	all AB .0090
(AB)(Ab) × (ab)(ab)	.167	....	50% A .0835	....	50% AB .0835
(AB)(aB) × (ab)(ab)	.044	....	....	50% B .0220	50% AB .0220
(AB)(ab) × (ab)(ab) ⎫ (Ab)(aB) × (ab)(ab) ⎬	.780	25% O .195	25% A .1950	25% B .1950	25% AB .1950
Total AB × O	1.000	.1950	.2785	.2170	.3095

The four columns, O, A, B and AB, should thus in linkage show approximately 20 per cent., 28 per cent., 22 per cent. and 30 per cent. of the offspring, respectively. The relative proportions of groups A and B would change somewhat with the race, A being greater in European races and B greater in Asiatic races. Linkage would not, however, produce large numbers of individuals in the two middle columns (non-crossovers) and small numbers in the two outside columns (non-crossovers), as the investigators cited above have thought.

There is thus seen to be no basis for Bauer's hypothesis of linked factors as applied to the human blood groups.

Two other suggestions of different workers may be briefly mentioned.

Wiener, Lederer and Polayes (1930), after reporting the results of the examination of the bloods of 1,334 mothers and 1,462 children, in which they find complete agreement with the hypothesis of triple allelomorphs, suggest a hypothesis to be used if it is necessary to explain exceptions. This hypothesis, which the authors probably do not advance very seriously, assumes the presence of 4 allelomorphs, O, A, B and a new factor D, which determines the presence of both agglutinogens in the cells and thus acts as AB. This can quickly be shown not to explain the recorded exceptions, however, and need not be taken seriously.

Levine has suggested to the writer and to other workers the possibility of non-disjunction as an explanation of the exceptional cases. If I can be convinced of actual exceptions, it is my belief that non-disjunction would be the most reasonable explanation for them. I have yet to find or to be shown a bona-fide exception, however, to the hypothesis of triple allelomorphs, and there can seem to be no reasonable doubt that this hypothesis must stand as the basis of the inheritance of the human blood groups. It is possible that it may be modified by the very occasional occurrence of the phenomena of non-disjunction.

LITERATURE CITED

Bauer, K. H.
    1928. *Klin. Wochenschr.*, 34, 1,588.
Bernstein, F.
    1925. *Zeitschr. f. Abst. u. Vererbungsl.*, 37, 237.
Castle, W. E.
    1930. ''Genetics and Eugenics,'' 4th Ed, Harvard Univ. Press, Cam-
                bridge, Mass.
Gates, R. R.
    1930. ''Heredity in Man,'' Macmillan, New York.
Kliewe, H., and Nagel, R.
    1927. *Klin. Wochenschr.*, 6, 2,332.
    1928. *Klin. Wochenschr.*, 7, 406.
Snyder, L. H.
    1929. ''Blood Grouping in Relation to Clinical and Legal Medicine,''
                Williams and Wilkins, Baltimore, Md.
Thomsen, O.
    1928. *Compt. rend. Soc. de Biol.*, 98, 1,270.
Wiener, A. S., Lederer, M., and Polayes, S. H.
    1930. *J. Immunol.*, 18, 201.

A LAMARCKIAN EXPERIMENT

PROFESSOR HAROLD S. COLTON
UNIVERSITY OF PENNSYLVANIA

INTRODUCTION

AN experiment begun in 1925 to test out the Lamarckian hypothesis in evolution ran for five years and was abandoned. The Lamarckian hypothesis has many followers, it is superficially reasonable, difficult to disprove, yet it is equally difficult to find facts for its support; facts without a flaw. As Lamarck defines the hypothesis in his "Philosophie Zoologique" four factors are emphasized:

In every animal which has *not exceeded its term of development*, the more frequent and *sustained use* of any organ gradually strengthens the organ, develops and enlarges it and gives it strength proportional to the *length of time* of such use; while the constant *lack of use* imperceptibly weakens it, causing it to become reduced, progressively diminishing its faculties, and ends in its disappearance. . . . Everything which nature has caused individuals to acquire or lose . . . is preserved *by heredity* and passed on to the new individuals which descend from it. (Packard's 1901 Translation.)

If we summarize the factors mentioned above we find:

1. Sustained use or disuse of parts increases or decreases an organ.
2. During the growth period of the organ.
3. Over a period of time; which is interpreted to mean over a series of generations.
4. The changes are inherited.

In the investigation which is described below, factor (1) and (2) can be demonstrated as true. Factor (3) was not tested sufficiently (five years or six generations) and factor (4) was negative. An orthodox Lamarckian would rightly criticize these experiments because factor (3), the time factor, was too short. As the experiment was carefully controlled, as far as it went, it is thought that the conclusions should be recorded.

Material and Methods

Albino rats from the Wistar Institute Experimental colony strain were selected as the medium for the experiment because the generations are short, their vital statistics very complete (Donaldson, 1924), methods for their care available (Greenman and Duhring, 1923), and the dimensions of the bones were capable of statistical treatment.

From these rats the fore limbs were removed, under ether, four to twelve days after birth (Colton, 1929). The rats were raised in special cages which allowed a six foot run (Colton, 1929). The biped rats so produced were bred for six generations.

In the first generation one half of each litter was not operated upon and these constitute the control. Although it was planned to kill the rats when 150 days old, various conditions interfered with this plan in generations beyond the first. Most of the members of the fifth generation were over a year old when killed, because every chance was given them to produce offspring; and it was only when the reproductive period of the females was over that they were killed. This may account for some of the discrepancies between the measurements of the fifth generations and those of the earlier ones.

When the rats were killed the skull and both hind legs were dissected off, boiled in 2 per cent. Star-Naphtha, and the bones cleaned. These were dried at room temperature and then stored away in glass containers until measured.

When the plans for the experiment were under consideration, it was thought that the statistical methods would be simple. The difference between the means of the dimensions of organs of the biped rats and those of the control was to be recorded; and, as a test of reliability of these differences, the differences were to be divided by their standard error. If a difference be over two times the standard error then the difference was to be considered significant.

But all sorts of difficulties arose because rats through life never stop growing and all that time the relative proportions of organs are normally changing. A correction based on body length was first considered; so, because of the unreliability of this dimension, the measurement of a soft part which can never be accurate and which, after the first measurement, can not be verified, the skull length was taken as a base.

When the program was planned, in 1925, the author expected to carry the experiments through at least ten generations. Although the rats bred well through four generations, yet in the sixth only four rats were produced and these like most of the fifth generation refused to breed. It is thought that some dietary lack in the autumn of 1929 was responsible for this failure to breed. No visible sign of a dietary lack was evident. The rats were in fine physical condition. Three female specimens of the fifth generation were submitted to the Wistar Institute for examination. Dr. Donaldson reported as follows:

> 682—left ovary infected and enlarged—other normal.
> 683—lung infected, not very badly: probably pneumonia.
> 672—ovaries seem normal; may be tiny fetus in left horn of uterus near lower end.
> 673—left ovary and tip of uterus full of pus. Right ovary seems normal; capsule in uterus just beneath ovary appeared hard and granular like cancerous tissue.

It will not be necessary to report in detail the methods used in the measurements of the bones as these were fully discussed in a previous paper (Colton, 1929).

The following measurements were made on each rat:

1. Body length—nose to anus—measured in a special trough.

2. Total length—nose to end of tail—measured in a special trough.

3. Skull length.

4. Skull breadth.

5. Femur length—right and left, right only used in tabulation unless right was injured in preparation and then the left was substituted.

6. Tibia length—right and left, right only used in tabulation unless the right was injured in preparation and then the left was substituted.

7. Tibia breadth.

8. Mesial bend of fibula. In the paper (Colton, 1929) an error is made in reporting the method used. On page nine and in Fig. (1) H, the measurement should be from
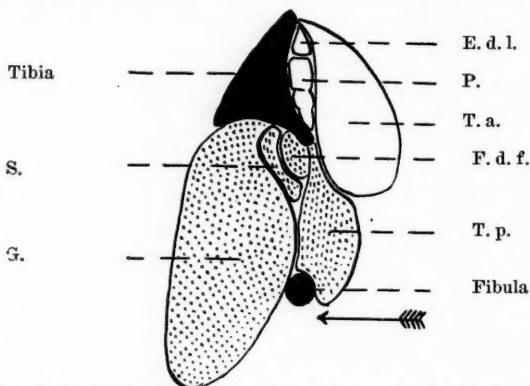

Fig. 1. Diagrammatic cross-section of the right hind leg of an Albino rat showing the relations of the bones and muscles. The bones are black, the flexor group of muscles stippled and the extensor and peroneal groups plain. The arrow points to the direction which the fibula is shifted in the biped rats.

E. d. l.   Extensor digitorum longus.
F. d. l.   Flexor digitorium longus and fibularis.
G.   Gastrocnemius medialis, Gastrocnemius lateralis, and Plantaris.
P.   Peroneal group.
T. a.   Tibialis anticus and extensor hallucis.
T. p.   Tibialis posticus.

the outer edge of the tibia to the outer, not inner edge, of the fibula.

In compiling the tables of results which are reported below, the bones of each operated rat were compared with a control of equal skull length. This was accomplished through the preparation of curves of femur length on skull length, tibia length on skull length, etc.

By means of these curves, from the femur length of the operated rats, the femur length of the control rat of equal skull length is subtracted.

The same process is repeated for tibia length, for tibia breadth and for mesial bend of the fibula. From these differences, means, standard deviation and standard errors are computed by ordinary methods. These are recorded in Table I. If a difference is twice the standard error it is considered significant.

TABLE I

Bone	Generation	No.	Mean of differences between biped and control	Standard error of difference	Difference divided by standard error
Femur l.	F 1	115	.73 mm.	± .078	9.3
	F 2	18	.69	± .238	2.8
	F 3	15	.56	± .199	2.9
	F 4	26	.71	± .128	5.5
	F 5	25	.06	± .139	No Sig.
Tibia l.	F 1	115	.68	± .088	7.9
	F 2	18	.42	± .098	4.2
	F 3	27	.47	± .121	3.8
	F 4	27	.74	± .145	5.0
	F 5	26	.42	± .081	5.2
Tibia br.	F 1	114	.18	± .021	8.5
	F 2	16	.38	± .115	3.3
	F 3	15	.09	± .042	2.1
	F 4	27	.26	± .058	4.9
	F 5	26	.03	± .029	No Sig.
Fib. mesial bend	F 1	110	.48	± .032	14.9
	F 2	17	− .02	± .068	No Sig.
	F 3	15	.25	± .081	3.1
	F 4	27	.51	± .021	2.4
	F 5	26	.52	± .049	10.6

An inspection of Table I shows what is well known; viz., a sustained use of an organ increases the size of the organ during the growth period. The differences of the first generation between the operated and control are

overwhelmingly significant. A further inspection of Table I shows that there is no progressive change to the fifth generation. Therefore, there is no indication that acquired characteristics are inherited.

Results

An inspection of Table I confirms the conclusions of the earlier paper on the difference between the first generation of bipeds and the control. It has an advantage over the earlier results, for it is based on over twice as many rats. It is seen that the femur is, on the average, .73 mm longer than control of same skull length, tibia .68 mm longer than control, tibia breadth .18 mm longer than control. The mesial bend of the fibula is .48 mm more than control. Therefore, a sustained use of these organs increases the size during the growth period. This is, of course, a well known phenomena.

To see if the changes in the bones were due to increases in the muscles, two experiments were performed in which certain muscles of the lower legs were dissected out and weighed wet. In the first experiment, 38 rats biped and 30 control.

The systems of muscles selected were:
- A. Flexor group.
  - 1. Gastrocnemius medialis, gastrocnemius lateralis, plantaris and soleus, dissected out and weighed as one.
  - 2. Flexor digitorium longus, and flexor digitorium fibularis weighed as one.
  - 3. Tibialis posticus.
- B. Extensor group.
  - 4. Tibialis anticus and extensor hallucis weighed as one.
  - 5. Extensor digitorium longus.
- C. Peroneal group.
  - 6. Taken out together, weighed as one.

The weight of each muscle was computed as a percentage of the sum of all the muscles of the lower leg. It will be noted in Table II that in the case of the extensor, peroneal groups and the tibialis posticus increased while the flexor system except the tibialis posticus tended to decrease.

TABLE II

Muscles	Difference in percentage between biped and control	
	60 days	150 days
Gastrocnemius, plantaris, and soleus	− .6	− 1.3
Flexor digitorium longus and f. d. fibularis	—*	− 3.7
Tibialis posticus	+ 39.7†	+ 9.4
Tibialis anticus and extensor hallucis	+ 1.1	+ 2.4
Extensor digitorum longus	+ .8	+ 1.1
Peroneal group	− 5.1	+ 1.9
Number of Rats operated	34	38
Number of Rats control	27	30

* In the case of the small rats it was next to impossible to separate the flexor digitorum longus and flexor digitorum fibularis from the tibialis posticus in a manner sufficiently uniform for statistical treatment. The flexor digitorum longus and f. d. fibularis have not been considered because the figures were so variable.

† In the small rats, part of the flexor digitorum fibularis and f. d. longus adhered to the tibialis posticus. That accounts in part for the large difference.

The experiment was repeated with 60 day old rats, 34 rats operated and 27 rats control, with results similar to the 150 day old rats, with the exception that the peroneal group of muscles showed a decrease. In the small rats it was difficult to separate the flexor digitorium group, fibularis and longus, from the tibialis posticus; so, as the results were irregular the two were added together.

Taking everything, all in all, it is important to note that the tibialis posticus and anticus on the lateral side of the fibula increase while the flexor group on the mesial side of the fibula decrease. This difference in the mus-

cles may account, in part, for the mesial bend of the fibula.

In an earlier report (Colton, 1930) it was shown that an angular bend in the tibia was correlated with the fact that, in walking, the feet of biped rats are spread apart, an adaption to greater stability. A mesial bend of the fibula would be caused by the bend in the tibia. Two factors then explain the bend of the fibula.

Conclusion

1. Although change of habit will change the proportion of the bones of the legs of rats, there is no evidence that over a span of six generations that the changes are inherited.

2. The mesial bend of the fibula seems to be due to a relative increase in the tibialis anticus and tibialis posticus, as well as a bend in the tibia, an adaption to greater stability.

3. As far as the Lamarckian factor in evolution is concerned the experiments are negative.

BIBLIOGRAPHY

Colton, H. S.
   "How Bipedal Habit Affects the Bones of the Hind Limb of the Albino Rat," *Jour. Exp. Zool.*, 53: 1–11.
Colton, H. S.
   1930. "Biped Habit," *Scientific Monthly*, 31: 81–85.
Donaldson, H. H.
   1924. "The Rat," *Memoirs* of the Wistar Institute of Anatomy and Biology, No. 6, p. 469.
Greenman, M. J., and Duhring, F. L.
   1923. "Breeding and Care of the Albino Rat for Research Purpose," Wistar Institute, p. 109.
Packard,
   1901. "Lamarck; the Founder of Evolution, His Life and Work, with Translations of His Writing on Organic Evolution."

THE ANTIQUITY OF INSECT STRUCTURES

PROFESSOR T. D. A. COCKERELL AND NORMA LeVEQUE

The University of Colorado

Any warm night in summer time, one may observe those minute Diptera known as punkies, which are so small that they pass without difficulty through the mosquito screen. Mere specks to the naked eye, they are nevertheless more complicated than any automobile, more efficient in flying than any aeroplane. They have muscles, nerves, a blood system, connective tissue, a reproductive system, and a marvelously complex exoskeleton. Their kind existed millions of years before man developed, and who can say that they may not continue to flourish after he has disappeared? Such marvelous little machines are of enormous antiquity, coming down through the ages in variously modified types, but with surprisingly little change in essential characters.

We are all interested in diversity, in progress, in evolution. But it is useful to consider also the stability of living things, the extraordinary fact that in them the most complex chemical substances known retain through vast ages their essential identity. The mammals, which have undergone so many remarkable developments during Tertiary time, are really exceptional. The lower vertebrates, and especially the invertebrates and plants, show a much slower rate of evolution, though it is possible or probable that they too had their time of rapid evolution in the remote past. Considering all the evidence, we are inclined to formulate some general statements, thus:

(1) The rate of evolution in any large group is not uniform; there are periods of relative stability, and periods of comparatively rapid change.

(2) Tertiary time has seen rapid change in the mammalia, but not in the insects, mollusks or flowering plants.

(3) Among the insects, we do indeed find many extinct genera in Tertiary rocks, but these are usually rather closely related to those now living, and the indications are that in at least the majority of cases they have died out, leaving no descendants.

(4) Every new collection of Tertiary insects shows us extinct species of genera still living, so that we are constantly pushing our records of these genera backward into the past, and increasing our estimates of their age. The same, of course, applies to the families.

(5) It is, of course, true that innumerable modern genera are unknown as fossils, but this need not be taken to suggest that they are of recent origin. Considering the small number of fossil insects, compared with those known living, we can not expect to find complete faunae for any periods in the past.

(6) Genera are of different degrees of distinctness. Some, which differ only by a vein in the wings, or a joint in the palpi, may indeed be very modern; for we can often observe these changes taking place as aberrations in existing species. Such genera, having no very fundamental distinctive characters, are likely to be more or less artificial. That is, species showing the characters may have arisen independently a number of times.

(7) Positive evidence of the extinction of genera in various regions is afforded by the fossils. Thus the tsetse flies (*Glossina*) have entirely died out in America, leaving no descendants. The family Nemopteridae once lived in North America, but has now disappeared from this continent.

(8) Probably the best evidence concerning the actual evolution of insect genera in Tertiary time may be derived from oceanic islands, especially when it is possible to form some idea of their antiquity. The Hawaiian fauna is especially instructive in this respect.

In order to present concrete evidence, we offer descriptions of several quite diverse insects, collected by Pro-

fessor Junius Henderson and Mr. John Byram in the rocks of Green River age in western Colorado. This deposit is Eocene, and is the oldest known, which contains an essentially modern (that is, like the modern) insect fauna of any size. The examples selected belong to five different orders.

LEPIDOPTERA

Tillyard has shown that the supposed Mesozoic Lepidoptera do not belong to that order. In the Tertiary rocks, Lepidoptera are scarce, owing to the fact that they are less likely to be preserved in recognizable form than any other winged insects. There are various records from the European Oligocene, and in America from the Miocene of Florissant. A beautifully preserved moth comes from the Oligocene of the Isle of Wight, and was described by Butler in 1889 as *Lithopsyche antiqua,* a new genus of Euschemidae. It has no striking peculiarities, but combines the characters of several living genera, which at the present day inhabit the Malay region. Were the climate such as to permit these moths to live to-day in Britain, the British genus or genera would most probably differ from those of the Malay Archipelago. Thus we need not suppose *Lithopsyche* to be the ancestor of *Calospila, Craspedopsis* and *Mniocera,* but merely that it died out in Europe when the environment changed. It does not appear evident that *Lithopsyche* is any more primitive than its living representatives. There is, however, a certain amount of evidence that the more primitive or the less specialized Lepidoptera, of families still existing, were proportionately more numerous in the faunae of Tertiary time than at present. Thus the senior author has in recent years had occasion to describe two new genera of fossil moths, one from the Oligocene of the Isle of Wight, the other from the Miocene of Florissant. Both are Cossidae, a family which all lepidopterists now place low in the series. In modern faunae, the chances would be very greatly against any two moths,

thus accidentally fossilized, being both Cossidae. In the Burmese Amber, which probably comes from low down in the Tertiary, a single moth has been preserved, and it belongs to the exceedingly primitive genus *Micropteryx*. Nevertheless, such moths still live in various parts of the world, so the fossils take us no nearer to the stem of the Lepidoptera than the oldest existing types.

With the possible exception of the specimen from Burmese amber, the moth now described appears to be the oldest Lepidopterous insect so far known.

Chionaemopsis new genus

Anterior wing only known. A rather small moth, the shape of the wing, and the pattern of dark cross-bands, strikingly like that of the genus *Chiqnaema,* which has numerous species in the tropics of the Old World. The venation, however, is conspicuously different, though in the fossil it is not as well preserved as could be wished. Another similar insect of quite different relationships is the Australian *Chrysonoma argutella* Zeller, of the family Oecophoridae. This has, as in the fossil, four vertical dark bands crossing the wings, the fourth forked above to make a Y. The venation of the Oecophoridae also agrees much better with the fossil; thus in the course of the subcosta, the radius forking before the middle of the wing, and its lower branch (radial sector) again forking in the region of the third band. In the figure, every effort has been made to show the venation of the fossil as it is, but it is so faint in many places that a really adequate drawing can not be made. It is not possible to make out the definite outline of scales, but small objects scattered over the dark areas appear to represent them, and there is an evident fringe.

The same pattern of four cross-bands appears also in *Iphierga* of the Tineidae, and there are reasons for believing it to be a very ancient one.
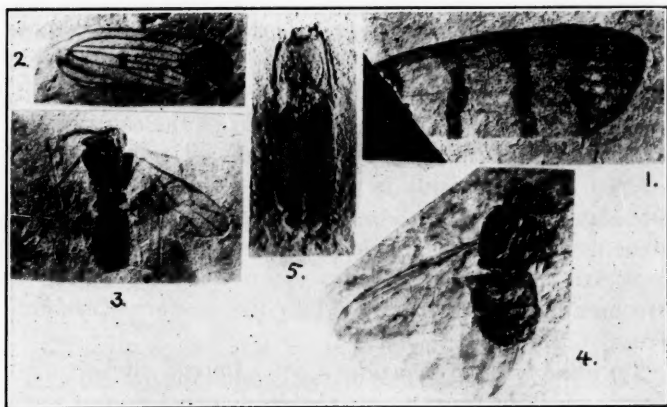
CHIONAEMOPSIS QUADRIFASCIATUS n. sp.

Anterior wing about 13 mm long (base missing) ; from first band to apex 10 mm; width nearly 5 mm; four dark cross-bands, the fourth forking near costa, as shown in figure.

Green River Eocene, Colorado.   Station 16, on Parachute Creek, 1923 (Henderson and Byram).

HOMOPTERA (FULGOROIDEA)

Protoliarus amabilis n. sp.   A beautifully preserved upper wing or tegmen, showing every detail of the venation and marking.   The wing is 7.6 mm long and 2.8 mm wide, thus considerably larger than *P. humatus* Cockerell. It also differs in the costal nervure, and in having the tegmen more broadly and obtusely rounded at end.   This genus closely resembles in a general way many now living, but its exact relationships are rather obscure, as has been explained in Proc. U. S. National Museum, Vol. 64, Art. 13 (1924) p. 8.

PHOTOGRAPHS

1. *Chionaemopsis quadrifasciatus.*   3. *Tryphon amasidis.*
2. *Protoliarus amabilis.*   4. *Chilosia scudderi.*
5. *Adelocera perantiqua.*

For the photographs we are indebted to the kindness of our colleague Dr. Ross Whitman.

Superficially the insect resembles *Oliarus,* and the size agrees with the fossil *Oliarites terrentula* (Scudder), but it is distinct by the clouded wings (wholly clear in Scudder's species) and the details of the venation.

The stem of the media before the first fork (where there is a vertically elongate dark spot) is about 1.9 mm long; from the fork to the end of the cell of which it is the base is 2.7 mm; the oblique vein to cubitus meets the fork of the latter.

In the existing fauna, these Fulgoroids present an amazing complex of related genera, new ones being continually discovered. It does not appear evident that *Protoliarus* is an ancestor of any of them, but the climate of the Rocky Mountains has changed and the numerous Fulgoroids, of tropical aspect, have disappeared.

Green River Eocene; Station 16, Parachute Creek, Colorado, 1923 (Henderson and Byram).

Hymenoptera (Ichneumonidae)

When we consider the numerous adaptive peculiarities of the parasitic Hymenoptera, and their often remarkable habits, it is natural to suppose that they are of relatively recent origin. This is not at all the case, and the Ichneumonidae of the Eocene are essentially at the same evolutionary level as those of modern times. The species now offered as an example is placed in *Tryphon,* as understood in the broadest sense, because the fine details of structure relied on for generic characters at the present time are not visible. Had we the complete insect, it presumably would fit easily into the modern classification.

Tryphon (s. lat.) *amasidis* n. sp. Length slightly over 6 mm, width of thorax 2 mm, of abdomen 1.6 mm; anterior wing 5 mm long. Wings hyaline, with pale nervures and stigma; antennae very pale; legs practically colorless as preserved; head and thorax dark, probably originally black; abdomen brown, the margins of the tergites

distinctly infuscated. Anterior wings with areolet elongated, four sided, the apical side smallest; lower end of basal nervure basad of nervulus by a distance fully equal to half length of latter. The peculiar shape of the thorax appears to be due to crushing. Compared with typical *Tryphon* the areolet is much longer (in *Tryphon* often practically square) and the basal nervure is much more remote from the nervulus. Another striking feature is the extremely oblique nervulus (the lower end apical), much in the manner of *Tryphon rutilator* (L), which is the type of the genus *Tryphon*. The species from the Florissant Miocene, described by Brues under *Tryphon,* seem to be doubtfully congeneric. In this key he separates *T. cadaver* from *T. peregrinus* by the shape of the stigma, but the latter, as figured, does not possess the kind of stigma indicated in the key. Of the Florissant species, ours appears to be nearest to *T. cadaver,* but that is smaller and has a much shorter marginal cell. Brues says *T. cadaver* has an areolet, but the figure shows none. In the key to Isle of Wight (Oligocene) Ichneumonidae, *T. amasidis* runs directly to *Lithapechtis fumosus,* which has quite the same kind of areolet, but differs in many respects.

The insect was presumably parasitic on some sawfly, and so is named after a genus of sawflies present in the Green River shales.

Green River Eocene, Station 26, Roan Plateau, 1923 (Henderson and Byram).

DIPTERA (SYRPHIDAE)

Chilosia scudderi n. sp. Length 7 mm; width of abdomen not quite 3 mm, length of anterior wing about 7.2 mm; head and thorax black; wings hyaline, slightly greyish; abdomen broad and short, pallid, with the hind margins of the tergites broadly infuscated; legs pallid so far as visible. The following measurements are in microns: length of costal cell 2860; base of costal cell to
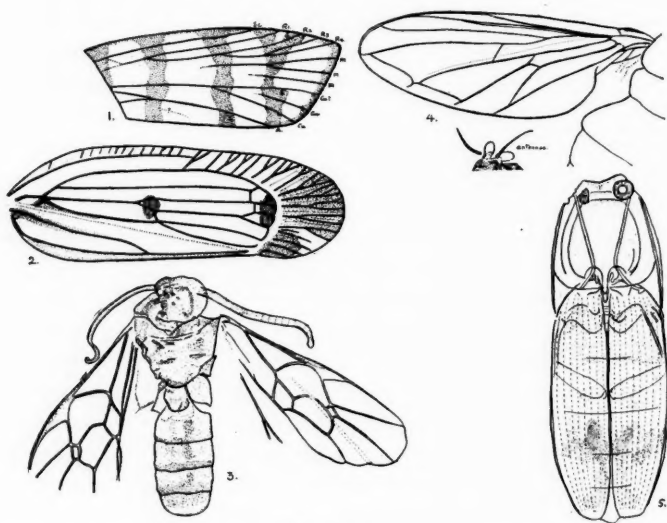
DRAWINGS

1. *Chionaemopsis quadrifasciatus.*   3. *Tryphon amasidis.*
2. *Protoliarus amabilis.*   4. *Chilosia scudderi.*
                5. *Adelocera perantiqua.*

end of subcostal 4900; length of anterior cross-vein 400; anterior cross-vein to base of first submarginal cell 900, to base of discal 800, to apex of discal 1960; last posterior cell on axillary 460; length of arista 1000.

This is evidently Scudder's *Chilosia* sp. figured in Tertiary Insects of North America, plate 9, f. 26, and discussed on p. 561. Scudder's specimen came from Green River, Wyoming.

This appears to be congeneric with *C. miocenica* Cockerell, from the Miocene of Florissant, but the marking of the abdomen suggests that it is not a genuine *Chilosia*. However, the Asiatic *C. plumbiventris* Brunetti has black bands on the abdomen.

The fly is in no sense more primitive than many now living.

Station 22, Roan Plateau, Colorado, 1923 (Henderson and Byram).

Coleoptera (Elateridae)

Adelocera perantiqua n. sp.. Length 8.5 mm, width across elytra 3 mm; color pale (eyes black), with two suffused dark marks on each elytron, giving the effect of broad transverse dusky bands.   The elytral striae are as usual, and the prosternal spine is long and acute.   The elytra are about 5.5 mm long.   Antennal grooves distinct.

It is about the size of the living *A. discoides* Web., but the blotched elytra rather suggest *A. marmorata* Fab. The elytral pattern and general appearance are suggestive of our common *Drasterius elegans* Fab., which, however, belongs to a different group.

Adelocera has many species in the Nearctic and Palaearctic regions to-day.   The European *A. fasciata* (L.) has the elytra much as in the fossil, but it also has spots on the thorax.

Station 20, Roan Plateau, Colorado, 1923 (Henderson and Byram).

SOME FACTORS AFFECTING THE DISTRIBU-
TION OF AND VARIATION IN NORTH
AMERICAN ECTOPARASITES

DR. H. E. EWING

BUREAU OF ENTOMOLOGY, U. S. DEPARTMENT OF AGRICULTURE

THE distribution of the groups of higher vertebrate animals and their variation and speciation in Nearctic North America have long been subjects for fruitful research. Associated with these higher vertebrates are host-infesting ectoparasites of several groups, the distribution of which is dependent to a greater or less degree upon the distribution of their hosts. Their distribution, therefore, offers a problem somewhat more complex than that of their free-living hosts.

Dr. Karl Jordan (1929) has presented a most excellent paper on the problems of distribution, variability and variation in North American fleas. He found that there were 131 described species in America north of Mexico. This number probably represents at least two thirds of all those that exist in this territory.

The outstanding fact in regard to the relationship of distribution to variation is that the latter, as expressed in numbers of genera and species, is far more pronounced in the western part of the continent than in the eastern part. Dr. Jordan gives the key to the situation in the following words: "Among the 31 species so far found in the Eastern States there are 17 which also occur in the West or at least are there represented by special subspecies, leaving 14 which are restricted to the East, but probably extend on to the Central plains, at least in the North. Some of these 14 are purely Northern forms, being only known from New England and the neighboring States; others are of wider southward distribution. In the Western area, on the other hand, the number of indigenous species confined to the West, but partly de-

scending eastward into the foothills, is 90, more than six times as many as in the Eastern States.''

Dr. Jordan has discussed various factors which may have brought about this paucity of genera and species in the eastern part of the continent as compared with the western part. Among these are mentioned the fact that apparently but little collecting has been done in the southeastern part of the United States and also that the summer climate of the low levels of the East is adverse to fleas in general. Yet the chief reason for the differences in the diversity of the flea faunas of the East and West is attributed to glaciation. He states: ''In the glacial period life was practically destroyed in the Northern Atlantic States, whereas in the Pacific half of the continent glaciation was less complete, so that life could persist in many cases. This, no doubt, accounts to a large extent for the greater abundance of species in the West.''

The present writer, having collected and studied ectoparasites from nearly all sections of the United States, has long noted this greater diversity of the fauna in the West. It is not at all confined to fleas but appears to be more pronounced in this group than in the host-infesting mites, the biting lice, or the sucking lice. Yet in the ticks, a group in which much of the time of the individual parasite is spent detached from its hosts, there is a condition paralleling that found in the fleas. Thus in the genus *Dermacentor,* a genus particularly well represented in North America in comparison with the other continents, there are present in the western part of the United States nine species, while in the eastern part there are only three and only one of these is abundant.

The present writer would attribute the greater diversity of the flea and tick faunas of the West, first, to the presence of natural barriers in the form of high mountain ranges, and secondly, to the diversity of climate. Along the northern border of the United States there are

three closely related species of *Dermacentor,* so closely related in fact that they were at first confused with one another. One of these, *D. occidentalis* Neumann, is found only west of the Cascade Mountains; another, *D. andersoni* Stiles, is found only between the Cascades and the plains; and the third, *D. variabilis* Say, is found only east of the arid plains, except for an isolated area along the Pacific slope of southern Oregon and California. That this distribution is brought about chiefly on account of natural barriers and different climatic zones is strongly indicated. The Rocky Mountain spotted fever tick, *Dermacentor andersoni* Stiles, originally was confined to the area between the Rocky Mountains on the east and the Cascade and Sierra Ranges on the west; but since the introduction of domestic animals it has spread considerably to the eastward but has never crossed the plains. There can be but little doubt that large numbers of *D. andersoni* have been carried into the plains and to the eastward in the past, and also that large numbers of *D. variabilis* have been carried into all regions of the West. Consider the number of these ticks that must have been taken into the West by the pioneers with their horses and cattle to say nothing of their dogs. It is stated that at times some of the roads to the western states were clogged with hundreds or thousands of these immigrants and their domestic animals. Yet with these ideal conditions for the spread of the eastern *Dermacentor,* it does not seem to have obtained a foothold in the West except along the southern part of the Pacific slope.

Variation and speciation have, in fact, taken place to a much greater degree in the western part of North America in groups other than ectoparasites. For example Howell (1929) finds that in the chipmunks (*Tamias* and *Eutamias*) there is but one species with five races in the eastern part of the continent; while there are sixteen species representing sixty races in the western part. Then there is that classical example of the song sparrow.

According to recent authorities there are no less than thirty-three races, or subspecies, of this common North American bird. Yet of this large number only four races are found east of the Rocky Mountains; while California alone has ten resident races. Chapman (1920) and others attribute this condition of affairs, and doubtless correctly, to the presence of barriers in the West and to the great diversity of climate, which in turn is dependent largely upon these barriers.

The apparently uneven and unusual way in which the flea, *Pulex irritans* Linnaeus, has distributed itself in the United States has been mentioned by Howard (1896) and Jordan (1929). However, the distribution of this species has not been ascertained with sufficient accuracy in the past. The unusual thing about its distribution has to do rather with its sparseness in certain large areas than its absence in them. Yet certain areas exist in which re-peated search for this flea has failed to reveal its pres-ence. In order to get a more complete and up-to-date picture of the distribution of this species, there are here given in tabular form all locality records, based upon specimens determined by specialists, that are in the files of the United States National Museum and of the United States Bureau of Entomology, particularly in the Bureau's Division of Insects Affecting Man and Animals.

AUTHENTIC RECORDS OF THE OCCURRENCE OF *Pulex irritans* LINNAEUS
IN THE UNITED STATES

Arizona: Madera Canyon, Santa Rita Mountains, June 19, 1898; Omaha, July 28, 1928; Phoenix, May 10, 1930.

Arkansas: Imboden, 1928; Georgetown, April 27, 1921.

California: Lakeside; Alameda County; Carbon Canyon, Puente Hills, January 23, 1926; San Diego, December, 1896; Azusa, July 23, 1894; Redwood Creek, Humboldt County, June 17, 1903; San Fran-cisco, October, 1907; Humboldt County, December 1, 1927.

Colorado: Larimer County, November 15, 1926.

District of Columbia: Washington, June 25, 1909 (by W. L. McAtee).

Florida: Little River, November 30, 1912, and December 20, 1913; Quincy, June 7, 1915.

Illinois: Elmwood, July 2, 1914; Winchester, 1921; Kempsville, April 28, 1921.

Indiana: Frankfort, April 29, 1914; Greenwood, June 19, 1922; locality
(?), May, 1928.

Louisiana: Port Barrie, April 26, about 1915; Tallulah, May, 1918; Mound,
June 17, 1918; locality (?), May, 1928.

Massachusetts: Fall River, August 16, 1921.

Michigan: Locality (?), 1921.

Mississippi: Dundee, April 12–15, 1921.

Missouri: Gilliam, August, 1914; Mexico, April 11, 1921; Atlanta, June 21,
1921; Charleston, July 1, 1921; locality (?), May, 1928.

Nebraska: Locality (?), fall, 1926.

New Jersey: New Brunswick, September 18, 1916; Browns Mills, May 5,
1916.

New York: Springville, November 7, 1921.

North Carolina: West Raleigh, April 8, 1915.

Ohio: Cincinnati, June 28, 1915; locality (?), June, 1923.

Oklahoma: Wister, July 8, 1904.

Oregon: Portland, 1903; Albany, June 20, 1915; locality (?), October 23,
1928.

Pennsylvania: Oxford, June, 1912; Chadd's Ford, April, 1921.

Texas: Brownsville, May 7 and 18, 1904, July 6 and 9, 1904, and July 5,
1895; locality (?), February 10 and 20, 1897; Dallas, September 1,
1915 (questionable identification), May 27, 1905, April 1, 1907, May
4, October 16, October 18, and November 21, 1917, and June 14,
1918; Concan, December 7, 1917; Uvalde, May 31, 1918; College
Station, June 3, 1918; San Antonio, summer, 1919; Sandy Point,
April 17, 1920; Ft. Worth, April, 1927.

Virginia: South Richmond, July 6, 1917.

Wyoming: Locality (?), November 27, 1927.

In addition to these seventy-one records which come
from twenty-three states and the District of Columbia,
there are many others that are not based upon specimens
determined by specialists. These other records are from
the same general areas as are covered by the valid ones.
Doubtless most of them refer also to *Pulex irritans*
Linnaeus.

If we mark on a map the definite locality records, we
get a distribution as indicated on the accompanying
map. Each dot represents a known locality record.
In some instances definite records were obtained for cer-
tain states but not for a locality in the state. These have
not been marked on the map. Thus there is a record for
the state of Michigan, another for Nebraska, and also
one for Wyoming, but, since the locality is not known in
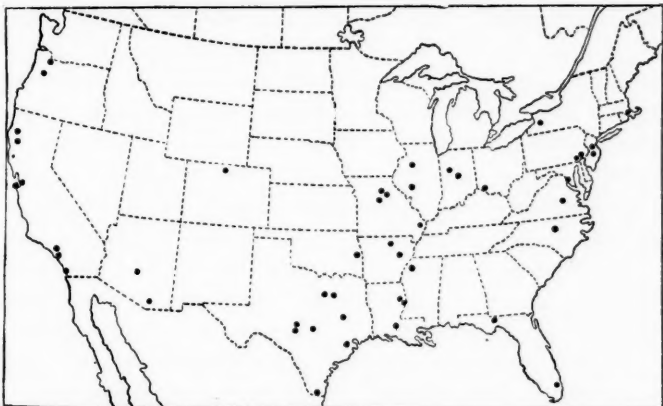
Map of the United States showing the distribution of *Pulex irritans* Linnaeus, as indicated by the records (based upon specimens) in the United States National Museum and the United States Bureau of Entomology.

each of these cases, the records could not be given on the map.

Three large areas are to be noted on the map from which we have no records or but very few of them. First, there is that large area between the Rocky Mountains on the east and the Sierra and Cascade Ranges on the west. This area includes the Basin States of Idaho, Nevada and Utah and much of the surrounding territory. Then there is the Great Plains area adjacent eastward of the Rocky Mountains. Finally, there is the southern coastal plain section of the South Atlantic States. Can the absence of records or scantiness of the same in this southern coastal plain section be attributed to lack of search for this flea, or to a probability that it is not being reported by entomologists in this as in the other three areas mentioned? There has been much collecting of ectoparasites in the Basin States area and also in the southern coastal plain section of the South Atlantic States. In fact from these areas both the Bureau of Entomology and the National Museum have an abun-

dance of records of other ectoparasites, including many flea species. In the Great Plains area but very little collecting has been done, and since this area is so sparsely settled one would not expect many reports from the inhabitants.

The absence of records from the Southern Appalachian section is probably due to the lack of collecting. At any rate, for the present, this section should be left out of consideration, as our knowledge of its ectoparasitic fauna is too limited. Dr. Jordan (1929) predicts that *Pulex irritans* will be found to occur in this section.

Since coming to Washington in 1919 the writer has been repeatedly reminded of Dr. Howard's (1902) statement that the fleas sent in for identification from the eastern cities are not *Pulex irritans* Linnaeus but either the cat flea or the dog flea. Not only has the present writer never received in all the eleven years of his connection with the Federal Bureau of Entomology a single specimen of *Pulex irritans* from the big cities of the East, but in his extended field work over a period of several years in the eastern parts of the states of Maryland, Virginia and North Carolina he has never taken a specimen of *Pulex irritans*.

About Washington, D. C., fleas are abundant but, as Dr. Jordan has noted, the number of species is not large. In the low swampy lands of eastern Virginia and North Carolina a month's survey work on ectoparasites by the writer and Charles East during the height of the flea season (July, 1928) revealed only four flea individuals. Two of these were *Echidnophaga gallinacea* Westwood on a rat, one was a species of *Ceratophyllus* on a rat, and the other was on a rat but escaped before being determined. Thus the absence of *Pulex irritans* in this section is attributed to adverse conditions that affect fleas in general. This does not hold in the least, however, as far as the District of Columbia is concerned, for the fleas are abundant in and about Washington. Thus out of thir-

teen specimens of one of our most common mammals, the white-footed mouse, *Peromyscus leucopus,* taken near Washington, eight were found to be infested with fleas; while out of eighteen specimens taken in the low swampy districts of eastern Virginia and North Carolina not a single one had as much as a single flea.

The scarcity of certain flea species in eastern Virginia and North Carolina may be attributed to the scarcity of their favored or normal hosts, but this explanation would not apply to the distribution of the majority of them. This section is very low and flat and most of it during some time of the year is flooded. The rainfall, however, both in total annual precipitation and in its seasonal distribution, is very nearly the same as at Washington, D. C. The surface water, however, does not drain off but accumulates in a soured mixture of peaty composition over much of the area. Experimentally such conditions have been approximated in the water treatment recommended by the Bureau of Entomology for the control of fleas, *i.e.,* the flooding of the infested soil with water, not once but several times, in order to destroy the flea larvae. Such treatments have proven effective against the human flea and dog and cat fleas.

What the writer has observed in regard to the paucity of fleas in his survey of ectoparasites of birds and mammals in the lowlands of eastern Virginia and North Carolina is in accord with the observations of Pearse in Africa. Pearse (1928) found that in Nigeria fleas were much more abundant in the section of that country where the climate was dry. He states: "Fleas flourish best on hosts which live in a dry climate and have a more or less permanent home, such as a burrow or a human habitation."

Although the moisture in the soil and the presence of surface water at frequent intervals on much of the area should account for most of the sparseness of the flea population in the section under consideration, yet there is also another factor to be considered. It is this: The

mammal population as a whole is inclined more to aquatic or semi-aquatic habits. In much of this section, instead of the cottontail rabbit, which avoids the water at all times, there is present the marsh rabbit, *Sylvilagus palustris palustris,* which is semi-aquatic, frequently taking to the water like a true water species.

Pulex irritans is a flea that thrives in association with man, although it may possibly not have man as its most favored host. It is frequently found on domestic animals, particularly pigs. Because of this close association with man and possibly with pigs also, but few of these fleas would be found in districts where human habitations are scarce. This would in part explain the absence or scarcity of *Pulex irritans* in the Great Basin and over the Great Plains. Excessive dryness would be particularly detrimental to the larvae of those species that normally infest nests of hosts that are built above the ground. This would not be true, however, of fleas on certain burrowing mammals, as they are known to thrive in desert conditions. Does *Pulex irritans* breed on any burrowing animal that occurs in our country? An answer to this question might help us understand better its distribution in the United States.

Summary

1. Ectoparasites that spend a part of their life detached from their hosts are affected not only by most of the factors that determine the distribution of their hosts but by many others that affect them as independent arthropods during their free-living periods.

2. The much greater diversity of species and genera in the western part of North America appears to depend fundamentally upon the presence of natural barriers in the form of high mountain ranges and upon the diversity of climate which is largely determined by the presence of these mountain ranges.

3. The distribution in the northern part of the United States of three closely related ticks of the genus *Dermacentor* is discussed.

4. Records of the occurrence of *Pulex irritans* Linnaeus in the United States are published, and the peculiar distribution of this parasite of man is considered.

5. The presence or abundance of *Pulex irritans* Linnaeus in any region of the United States appears to depend in part upon the following:

‧ (a) the proper moisture content in the soil and the absence of surface water on top of the soil during the period of its larval development,

(b) the absence of extremely low temperatures,

(c) the presence of human habitations,

(d) the presence of hosts other than man, these hosts for a particular region not being known at the present,

(e) the habits of all hosts (including man) in the region under consideration.

6. A study of the records of the occurrence and abundance of fleas in general and of *Pulex irritans* Linnaeus in particular would appear to indicate that the abundance of individuals is largely independent of the following factors;

(a) total annual precipitation,

(b) humidity during adult state.

LITERATURE CITED

Chapman, F. M.
    1920. "Handbook of Birds of Eastern North America," 530 pp., 24 pls., 136 figs. D. Appleton and Co., New York and London.
Howard, L. O.
    1902. "The Principal Household Insects of the United States," Chapter I, Mosquitoes and Fleas, pp. 9–31, figs. 1–6, *Bull.* No. 4, n. s., U. S. Dept. Agr., Div. Ent.
Howell, A. H.
    1929. "Revision of the American Chipmunks." North American Fauna, No. 52, 157 pp., 10 pls., 9 figs., Bur. Biol. Surv., U. S. Dept. Agr.
Jordan, K.
    1929. "On some Problems of Distribution, Variability and Variation in North American Siphonaptera," *Trans.* IV Intern. Cong. Ent., II: 489–499, 11 figs.
Pearse, A. S.
    1928. "Arachnids Found on Nigerian Rodents and Insectivores," *Jour. Elisha Mitchell Sci. Soc.*, XLIII: 171–180.

SHORTER ARTICLES AND DISCUSSION

THE EFFECTS OF SELECTION ON EYE AND FOOT ABNORMALITIES OCCURRING AMONG THE DESCENDANTS OF X-RAYED MICE

In 1921 the writer and Bagg discovered certain abnormalities of the eyes and feet in mice. These particular genetic abnormalities have occurred only among the descendants of certain animals which as adults (non-pregnant females and males) were treated with light doses of X-rays over the whole body. A series of papers dealing with the various abnormalities from genetic and anatomical points of view are included in the bibliography of this note.

The object of the present communication is to record the results of selection within the abnormal race as it is at present being carried on. It should be recalled that whereas Bagg has at present animals descended without an outcross from the original abnormals, the mice which provide the primary data incorporated in this note are the result of either one or two outcrosses with a normal albino race followed by close (brother to sister) inbreeding and, in certain of the lines, selection of the abnormal individuals as parents. The ancestry of most of these
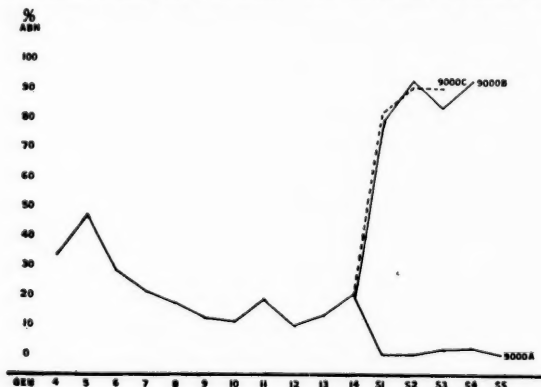
mice (the stock giving rise to lines 9,000A, 9,000B and 9,000C) is described in some detail by Murray (1928). The 9,000 lines are the result of two outcrosses of the abnormality followed by isolation through inbreeding. The other, line 700, is the result of only one outcross followed by inbreeding.

370

The derivation of the three 9,000 lines by selection is shown in Fig. 1. The first fourteen generations include the material described by Murray (Gen. 4–14); while the selection generations included in this note are designated as S_1–S_5. The details of the three lines will be given below.

Line 700, not shown in the figure, is the result of an attempt to isolate the eye abnormality from the various foot abnormalities. During the period from 1929 to the present year, inclusive, 355 animals have been recorded in it. These animals are distributed over four generations as follows, as regards eye abnormalities:

Generation	Right eye abnormal	Left eye abnormal	Both eyes abnormal	Total abnormal	Normal	Per cent. abnormal
I	35	33	2	70	9	88.6
II	50	36	11	97	7	93.2
III	41	43	17	101	6	94.3
IV	25	26	11	62	3	95.3
Grand total	151	138	41	330	25	92.9

In an earlier paper (1924) the writer and Bagg found by macroscopic examination approximately 81.8 per cent. of the progeny of abnormal-eyed individuals to be abnormal. It is evident that distinct progress is being made by selection in increasing the percentage of abnormal-eyed individuals among the progeny. There has also been, among the abnormals, an increase in the percentage of mice with both eyes abnormal. This percentage, in successive generations, has been 2.8, 11.3, 16.8 and 17.5, respectively.

There have been among the 355 mice only two foot abnormalities; ♂ 446 with right eye and right hind foot abnormal in the first generation, and ♂ 709 with left eye and right anterior foot abnormal in the fourth generation. The percentage of foot abnormals is thus 0.59. It is doubtful whether they will ever be entirely eliminated from the strain by selection, but their reduction to this point is interesting. Bagg (1929) reports in a population of 5,200 mice the occurrence of foot abnormalities in 432, or 8.3 per cent. Murray (1928) in 3,069 mice found 565 young with abnormal feet, a percentage of 18.4. There has, therefore, in the ''700'' stock, been definite progress towards decreasing the percentage of foot abnormalities.

The three "9,000" selection lines A, B and C were started for different purposes from a single original stock.

The line 9,000A was a reverse selection in the direction of the normal type. In the last five generations the following result has been obtained.

	Generation	Normal	Abnormal
A_1–S_1	....	44	0
A_2–S_2	....	40	0
A_3–S_3	....	55	1
A_4–S_4	....	107	2
A_5–S_5	....	43	0
	Grand total	289	3

In the total number only 1.03 per cent. is abnormal.

The three abnormal individuals are

♀ 1,088 normal eyes and abnormal left posterior foot,

♀ 1,262 normal eyes and abnormal left posterior foot, and

♂ 1,469 abnormal right eye and abnormal left anterior foot.

There is, therefore, in this line only one animal with abnormal eyes out of the total of 292. This percentage of 0.34 is interesting and in marked contrast to the 92.9 per cent. eye abnormalities obtained by selective breeding in line "700."

Line 9,000B is a selection for high number of foot abnormalities especially in the anterior feet. The last four generations have given 85.2 per cent. abnormal as follows:

Generation	Foot abnormality in anterior feet only	Both anterior and posterior foot abnormalities	Foot abnormality in posterior feet only	Total abnormals	Total normals
B_1–S_1	18	9	2	29	8
B_2–S_2	19	17	1	37	3
B_3–S_3	43	53	10	106	21
B_4–S_4	13	8	2	23	2
Grand totals	93	87	15	195	34 (85.1 Per cent.) (abnormal)

Line 9,000C is a selection for high number of foot abnormalities especially in the posterior feet. This line is less fertile than either line 9,000A or 9,000B. In the last three generations it has given a total of 136 animals distributed according to the following table.

Generation	Foot abnormality in anterior feet only	Both anterior and posterior foot abnormalities	Foot abnormality in posterior feet only	Total abnormals	Total normals
C_1–S_1	4	10	9	23	5
C_2–S_2	11	23	12	46	5
C_3–S_3	10	30	11	51	6
Grand totals	25	63	32	120	16

The total shows 88.2 per cent. of the animals with abnormal feet.

Line 9,000B shows 84.1 per cent. animals with anterior feet abnormal and 75 per cent. with posterior feet affected. Line 9,000C has 84.6 per cent. with abnormal anterior feet and 85.5 per cent. with posterior feet affected. Leaving out individuals that have abnormalities in both anterior and posterior feet, line B has 86.1 per cent. of its abnormals in the anterior feet only. Line C analyzed similarly shows 43.8 per cent. Some progress in the direction of the selection has been made but it is not as extensive or as striking as is the progress made in the total percentage of foot abnormalities. This for the two lines is 86.3 per cent. and is an interesting contrast with line "700" with 0.59 per cent. and line 9,000A with 1.02 per cent.

The distribution by feet of the abnormalities is also a matter of interest. Compared with the findings of Murray and of Bagg this is as shown in the following table.

It will be noted that Murray's figures and those given by Line 9,000B show a clear excess of left anterior feet; while Bagg's total shows an excess of left posterior feet. This demonstrates an interesting genetic difference which is very likely due to modifying factors in the two strains.

Author	Total number of abnormal feet	Right anterior	Left anterior	Right posterior	Left posterior
Murray, 1928	726	154	*277*	.132	163
Bagg, 1929	510	29	128	149	*204*
Little, 1931,					
Line 9,000B	421	126	*161*	63	71
Line 9,000C	284	68	75	75	66
Grand total	1941	377	641	419	504

Murray's mice and the line 9,000B mice show a total of 718 abnormal anterior and 429 posterior feet; while Bagg's mice show 157 anterior to 353 posterior feet abnormal. Line 9,000C totals 143 abnormal anterior feet and 141 abnormal posterior feet.

A comparison between right and left sides of the body shows the following results:

Author	Right	Left	Per cent. left
Murray, 1928	286	440	60.6
Bagg, 1929	178	332	65.1
Little,			
Line 9,000B	189	232	55.1
Line 9,000C	143	141	49.7
Grand totals	796	1145	58.9

From these figures it would seem that selection in line 9,000C has increased significantly the proportion of right foot abnormalities. There appears to be an inherent tendency in the figures of Murray, of Bagg, and of line 9,000B to produce a significant excess of abnormal left feet. This is to some degree being overcome in line 9,000C.

The above results demonstrate clearly

(1) That it is possible within the abnormal stock by selection to develop strains differing greatly in the percentage of animals showing eye abnormalities (92.9 and 0.34 per cent.);

(2) That it is possible within the abnormal stock to develop by selection strains differing greatly in the percentage of foot abnormalities (88.2, 85.1 and 0.59 per cent.);

(3) That it is possible by selection to develop within this material a strain high in eye abnormalities (92.9 per cent.) and low in foot abnormalities (0.59 per cent.);

(4) That it is possible by selection, but with more difficulty than in the foregoing cases, to progress in the direction of separating a line high in anterior foot abnormalities (86.1 per cent.) from a line high in posterior foot abnormalities (56.2 per cent.);

(5) That modifying factors appear to play an important rôle in determining the antero-posterior and right-left relationships and incidence of the abnormal feet.

C. C. LITTLE

ROSCOE B. JACKSON, MEMORIAL LABORATORY,
BAR HARBOR, MAINE

LITERATURE CITED

Bagg, H. J.
    1923. *Proc Soc. Exp. Biol. and Med.*, 21: 146–149.
    1924. *Proc. Soc. Exp. Biol. and Med.*, 21: 228–229.
    1924. *Am. J. Obst. and Gyn.*, 18: No. 2.
    1925. *Am. J. Anat.*, 36: 275–311.
    1929. *Am. J. Anat.*, 43: 167–219.
Bagg, H. J., and Halter.
    1927. *Anat. Rec.*, 37: 183.
Bagg, H. J., and Little, C. C.
    1924. *Am. J. Anat.*, 33: 119–145.
Bagg, H. J., MacDowell, E. C., and Lord, E. M.
    1925. *Anat. Rec.*, 31: 342.
Bean, A. M.
    1929. *Am. J. of Anat.*, 43: 221–246.
Little, C. C.
    1926. Annals of Clin. Med. V, No. 1.
    1928. *Surgery, Gyn. and Obst.*, January, pp. 155–158.
Little, C. C., and Bagg, H. J.
    1923. *Am. J. of Roentgenology* X: 975–989.
    1924. *J. Exp. Zool.*, 41: 45–91.
Murray, W. S.
    1928. Papers of the Michigan Acad. of Sci. Arts and Letters, X, pp. 509–587.

A POSSIBLE EXPLANATION OF THE APPARENTLY IRREGULAR INHERITANCE OF POLY-DACTYLY IN POULTRY

In a recent paper by Punnett and Pease (1929) the known facts concerning the inheritance of polydactyly in poultry were brought together and analyzed. It was fairly conclusively demonstrated that a single factor was responsible for the development of extra toes, and certain families gave results in close agreement with Mendelian expectation, with the extra-toed (E.T.) condition dominant over the normal four-toed (n.e.) condition. In many families, however, four-toed (n.e.) birds appeared in F_1 between homozygous E.T. and n.e. birds, and an excess of normal birds was found in F_2 and backcrosses. Where normal birds obtained in F_1 were tested, they were found to carry the E.T. factor.

These irregular results were ascribed to the effect of factors inhibiting the development of extra toes. The observation that a bird with extra toes might carry factors causing an excess of normal birds among his progeny made it difficult to devise a simple factorial scheme to explain all the results.

The object of the present note is to suggest that these facts are consistent with Fisher's (1928) theory that dominance is controlled by genetic factors.

On this view three main types of behavior may be expected:

(1) Normal Mendelian behavior with complete or nearly complete dominance of the polydactylous condition. This will occur when both parents carry factors favoring dominance of E.T. (hereafter referred to as dominance factors).

(2) Abnormal behavior with normal four-toed birds occurring in F_1, and normal birds in excess of the proportions expected when polydactyly is completely dominant, in F_2 and backcrosses and a corresponding proportion of normal birds behaving as heterozygotes. This will occur when one parent lacks some or all of the dominance factors. It may be either parent which lacks dominance factors, but heterozygotes lacking all the dominance factors should be four-toed (normal).

(3) Normal behavior, with complete or nearly complete dominance of the four-toed condition. This will occur when neither parent carries dominance factors.

The data assembled by Punnett and Pease may now be examined to see how far they fall into these classes.

(1) Normal behavior, with polydactyly dominant. The best example of this is the light Dorking × Buttercup cross made by Punnett and Pease (pp. 343–344). In F_1 15 chicks were obtained, all polydactylous. In F_2 out of 128 birds, 92 were polydactylous and 36 were normal. In a backcross to the Buttercup (normal) father there were obtained 20 polydactylous and 20 normal birds.

Bateson's White Leghorn × White Dorking material (quoted by Punnett and Pease, p. 348 and Table I) and some Silky crosses (Table II) gave comparable results.

(2) Abnormal behavior, with polydactyly incompletely dominant. Dorking × Brown Leghorn, and some of the Silky × Brown Leghorn crosses fall in this class. Records are given of F_1's and an F_2 of Dorking × Brown Leghorn (Punnett and Pease, Table I) and of F_1's, F_2's, and backcrosses to both parents, of Silky × Brown Leghorn and Recessive White (a type extracted from a Brown Leghorn cross) (Punnett and Pease, Table II).

TABLE I

FREQUENCY ARRAYS OF PERCENTAGE OF NORMAL BIRDS IN BROWN LEGHORN AND RECESSIVE WHITE CROSSES. REARRANGED FROM PUNNETT AND PEASE

Per cent. normals	0 to 4	5 to 9	10 to 14	15 to 19	20 to 24	25 to 29	30 to 34	35 to 39	40 to 44	45 to 49	50 to 54	55 to 59	60 to 64	65 to 69	70 to 74	75 to 79	80 to 84	85 to 89	90 to 94	95 to 99	Total
Brown Leghorn F_1		2	1																		3
×																					
Dorking F_2						1															1
(Brown Leghorn) F_1	4	1	4		1	1															11
and																					
(Recessive White) F_2						5	1	2	2	1	1		1						1		14
×																					
Silky																					
(F$_1$E.T.) × Normal										1		1	1	2	1	2	2	3			13
(F$_1$n.e.) × Normal												1			1		2	2			6
F_1 × Silky		5		2				1													8

To the true F_1's have been added families S60, S64 and S65. The polydactylous parents of these families were from a backcross of (Silky × Brown Leghorn) × Silky, and since they gave

less than 25 per cent. of normals when crossed with normals, they are assumed to be homozygous for the E.T. factor, but to lack some of the dominance factors. F_1's then give from 0 to 26 per cent. of normal birds.

Nineteen backcrosses of heterozygous E.T. to normal birds were recorded, in which the heterozygote was either an extracted polydactylous bird (13 families) or an extracted normal bird which carried the E.T. factor (6 families).   These gave from 40 to 86 per cent. of normal birds, and the families of which the heterozygous parent was normal gave on the whole a much higher proportion of normals than the rest.

Eight backcrosses of heterozygous E.T. to homozygous E.T. were recorded and gave from 0 to 35 per cent. of normal birds. In one case the heterozygote was normal in appearance, but none of its progeny was normal.

Fifteen F_2 families were recorded, and gave from 25 to 90 per cent. of normal birds.

Two heterozygous E.T. cocks from White Dorking crosses were bred both to Brown Leghorn hens and to hens of other breeds.

TABLE II

White Dorking × Indian Game			(White Leghorn × White Dorking) × (Indian Game × Brown Leghorn)		
Normal parent	Expt. No.	Per cent. normal progeny	Normal parent	Expt. No.	Per cent. normal progeny
Brown Leghorn		164	70	Brown Leghorn	168	61
White Leghorn	9	163	69	White Leghorn 565	167	40
do.	483	165	51	Buff Leghorn	169	52
do.	548	166	61	O. E. Game	170	50

Omitting the progeny of White Leghorn 9 (Expt. 163), breeds other than Brown Leghorn gave approximately 50 per cent. of normal birds, as expected when polydactyly is completely dominant, and Brown Leghorn gave considerably more than 50 per cent. of normal birds, as expected when some dominance factors are lacking.   White Leghorn 9 was the only bird tested of its breed which seems to have lacked dominance factors.

(3) Normal Mendelian behavior with the four-toed condition dominant.   Since all the polydactylous breeds which have been investigated gave a large proportion of polydactylous birds in F_1, when crossed with normal birds, families in which normal behaves as a dominant will be expected only very rarely unless dominance factors are eliminated by selection.

Hen 461 from Expt. 40 (Silky × Brown Leghorn) was a normal heterozygote which must have lacked most of the dominance factors.   She was used in Expts. S20, S43 and S58 (Punnett and Pease, Table II).   In Expt. S58 she was mated with a normal Recessive White cock, and in a family of 14, only 2 birds were polydactylous.   In Expt. S43 she was mated with a brother with an extra toe on one foot only, and in a family of 10 only one bird was polydactylous.   In Expt. S20 she was mated to an F_1 cock from a Silky × Brown Leghorn cross in which all F_1 birds were polydactylous, and which therefore presumably carried dominance factors.   In a family of 49 there were 14 normal birds, or 29 per cent.

In Davenport's Houdan experiments (quoted by Punnett and Pease, Table III) there were several crosses between heterozygous normal birds, and among these 86×83 (Table III B) gave 12 normal: 4 polydactylous; and 2324×936 (Table III B) gave 30 normals: 8 polydactylous, both very close to expectation in an F_2 with normal dominant.

Punnett and Pease classified their birds into "non-resistant" and "resistant" according to whether they gave progeny agreeing with Mendelian expectation (with polydactyly dominant) or differing from it.   The assumption made in the present note is that such "resistance" factors affect only the heterozygote.

J. B. HUTCHINSON

LITERATURE CITED

R. A. Fisher.
    1928. "The Response of the Wild Type to Recurrent Mutations,"
            AM. NAT., 62: 115; "Two Further Notes on the Origin of
            Dominance," AM. NAT., 62: 571.
R. C. Punnett and M. A. Pease.
    1929. "Genetic Studies in Poultry.   VII. Notes on Polydactyly,"
            Jour. Gen., 21 (No. 3): 341.

INTERGENERIC HYBRIDS BETWEEN GOSSYPIUM
AND THURBERIA

THE genera *Thurberia* and *Gossypium* are referred by Bentham and Hooker to the same tribe (Hibisceae) of the natural order Malvaceae. The chromosome number of the so-called Arizona wild cotton, *Thurberia thespesioides* A. Gray, has been determined by Dr. A. E. Longley (personal communication) as 13. The correctness of this number, which is the same as that of the cultivated Asiatic cottons, *G. stocksii,* and the wild Mexican cotton, *G. Davidsonii,* has been verified in this laboratory by J. E. Lans in acetocarmine preparations.

Thurberia thespesioides is morphologically very similar to *Gossypium,* and indeed was described by J. Miers (*Jour. Bot.,* xxxl, 330) as *G. lanceoeforme.*

Seeds of this species were obtained from Arizona through the kindness of Dr. T. Kearney and the resulting plants proved to be moderately well adapted to Trinidad conditions.

Attempts have been made to hybridize Thurberia with other species of Gossypium, and success has been attained in three instances.

(1) Gossypium stocksii ♀ × Thurberia thespesioides ♂

This cross was quite easily made, though the resulting plants were very weak and died just after the seedling stage after two or three leaves had been formed. The former species has deeply laciniated, while the latter has shallowly indented leaves. The hybrid possessed distinctly laciniated leaves, though it was not possible to state whether they were as laciniated as in Thurberia.

(2) Gossypium Davidsonii ♀ × Thurberia thespesioides ♂

Nine seedlings were obtained from three bolls. Brown glands appeared on the cotyledons and the latter rapidly developed an extensive necrosis leading to the death of all the seedlings before the second leaf was formed.

(3) New World Cultivated (Upland × Sea Island × Sea Island) ♀ × Thurberia thespesioides

One healthy plant of this cross was raised. The ♀ parent was Yy Pp Ss in composition (yellow corolla, yellow pollen, spotted) and Thurberia possesses a pale cream corolla, pale cream pollen and a petal spot. The hybrid plant was phenotypically yps,

which can be accounted for by assuming that the particular egg cell fertilized was yps. We should expect, however, that the hybrid would show dominance of spot from the Thurberia parent. That it does not may be provisionally explained if the ♀ parent carries two recessive spot genes and not one, since there is every reason to believe that there are, in New World cottons, two separate loci for S in different chromosomes. The present case would then be analogous to Bridges'[1] triploid Drosophilas, in which he found that the combination of two recessive genes with one dominant was sometimes phenotypically recessive.

A comparison of the main features of the Thurberia hybrid and its parents is placed below:

	NEW WORLD	THURBERIA	HYBRID
Leaf shape	Moderately laciniated	Deeply laciniated	Intermediate
Leaf lobes	Predominantly 5	3	4–5
Bract teeth	Present (10–14)	Absent	Present (5–8)
Internal boll hairs	Absent	Present	Present
Bract size	Large	Small	Small
Bract (external glands)	Absent	Present (3)	Present (3)
Boll size	Large	Small	Intermediate (nearer to Thurberia)

Another successful cross has been made using as the female parent a plant of the composition Upland × Sea Island × Sea Island × Sea Island. Seven plants were raised, closely resembling the hybrid described above, except that they had yellow corolla, yellow pollen and a petal spot. Attempts to cross pure Peruvian, pure Upland or cultivated Asiatics by Thurberia have so far been without success.

Sterility of hybrids. The pollen of the hybrids has proved none functional when applied to a wide range of types, including Peruvian, Upland, *G. Davidsonii*, *G. tomentosum*, Thurberia, *G. stocksii* and cultivated Asiatics. When selfed or when pollinated by pollen of the above forms, the capsules of the hybrid remain on the plant for a considerable period, but when mature show only shrivelled ovules devoid of embryos.

[1] Morgan, T. H., Bridges, C. B., and Sturtevant, A. H., "The Genetics of Drosophila," Bibliographia Genetica, Vol. II, 1925.

Taxonomic position of Thurberia thespesioides. A. Gray.
Since Thurberia has 13 chromosomes and will cross with (*a*) an
Asiatic species of Gossypium (*G. stocksii*) with 13 chromosomes,
(*b*) a New World species (*G. Davidsonii*) with 13 chromosomes,
and (*c*) cultivated New World cottons with 26 chromosomes,
there seems to be no valid reason for excluding it from the genus
Gossypium. We therefore propose to reinstate it in the genus
under the name, *Gossypium lanceoeforme* Miers.

S. C. HARLAND
O. S. ATTECK

GENETICS DEPARTMENT,
    COTTON RESEARCH STATION,
        TRINIDAD, B. W. I.

THE SPECTRUM ANALYSIS OF EYE COLOR IN DROSOPHILA

EYE color changes produced in Drosophila by gene mutation
have been studied extensively. The comparison of eye colors has
been made, however, by visual examination of the integrated
spectrum of light reflected from the eye. This makes the descrip-
tion of an eye color difficult and a quantitative comparison be-
tween eye colors virtually impossible.

It seems that the problem might be considerably simplified by
examining the wave-length distribution of the reflected light.
Then the comparison of two eye colors would be reduced to the
comparison of their wave-length distribution curves. An obvious
application would be to the study of such allelomorphic series as
that at the locus of white on the X-chromosome of *Drosophila
melanogaster.*

Certain difficulties at once suggest themselves: the small
amount of reflected light attainable from so small an object, the
deterioration in the eye during exposure to intense light and the
necessity of finding a method sensitive enough to show the small
differences which exist between certain eye colors. The present
investigation is of a preliminary nature and was undertaken to
ascertain the possibility of overcoming the difficulties mentioned
above.

The general method adopted was that of photographing the
spectrum and of plotting the density curve for the photograph
from microphotometer measurements. The eye to be examined

was severed from the head, pasted upon a piece of black paper in such a way as to prevent evaporation from the cut surface, and mounted on the stage of a compound microscope. Light from the hot pole of a carbon arc was passed through filters of water and blue glass and focused on the eye by means of a concave mirror. The eye was magnified 150x and focused on the slit of a large-dispersion spectrograph. Panchromatic plates were used to permit the study of the spectrum from 4000 to 8000Å. It was found that an exposure of from one to three minutes was ample and that no appreciable deterioration of the eye occurred in this time.

In this investigation two eye colors were compared, Red and Dilute-red. Dilute is a *dominant* eye color mutation obtained by radium irradiation. It has the apparent effect of slightly diluting red, vermilion and other eye colors. A more detailed account of its genetic behavior will be published later.

Some typical results are shown graphically in Figs. 1 and 2. The irregular shape common to all the curves is due to the varia-
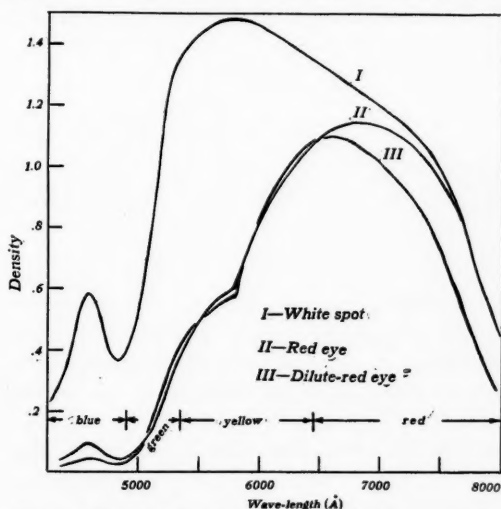

FIG. 1.   Complete density curves (1 minute exposure).

tion with wave-length of the sensitivity of the photographic plates. Curve I is from a spectrum obtained by replacing the eye by a spot of white paper, and may be used as a standard for comparison. As would be expected, both the Red and Dilute-red eyes show a strong selective reflection in the red, with considerable reduction at the short wave-lengths. A comparison of curves

II and III shows significant differences in the red and blue portions of the spectrum, the Dilute-red eye showing less reflection in these regions.   Curves IV and V of Fig. 2 were obtained with
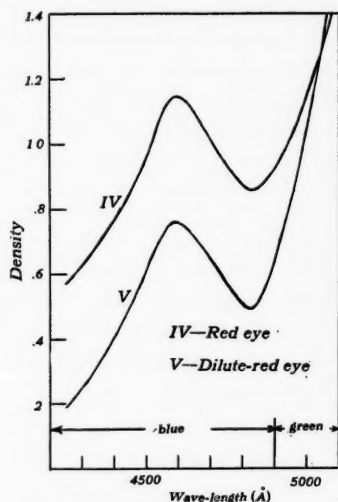

FIG. 2.   Partial density curves (3 minute exposure).

longer exposure and bring out more clearly the difference at the shorter wave-lengths.   The conclusion is that the effect of Dilute on the Red eye is a reduction of reflected light in two portions of the spectrum, the red and the blue.

This result is explained by histological examination of longitudinal sections of the ommatidia.   The normal Red eye contains two kinds of pigment granules, purple-red and ocher-yellow.   In the Dilute-red eye, the purple-red granules are found to be almost entirely absent from the distal portions of the ommatidia, and greatly reduced in number elsewhere.   This would account for the loss in intensity in the red and blue spectral regions.

In order that results obtained in different experiments may be comparable, it is necessary to make allowance for the unequal energy distribution in the spectrum of the light source and for the variation of the sensitivity of the photographic plates with wave-length.   This preliminary investigation has been sufficient, however, to show that the difficulties in the way of a quantitative study of eye color differences in Drosophila are not serious.

ELVENE W. VANATTA
L. C. VANATTA
WASHINGTON UNIVERSITY